Index Number

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Volume 59 DECEMBER, 1961 *Number* 6

To Honor
JOSEPH STOKES, JR., M.D.
in his 65th year

HIS STUDENTS AND COLLEAGUES HAVE CONTRIBUTED TO THIS ISSUE

Special Editorial Committee, T. F. McNair Scott, M.D., Chairman

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Harris and Harris

Virus-induced acquisition of metabolic function
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The Journal of Pediatrics *joins his students and colleagues on the event of his 65th year in the recognition of his contributions to pediatric medicine.*

THE JOURNAL OF
PEDIATRICS

DECEMBER 1961 *Volume* 59 *Number* 6

Address given on the occasion of a farewell dinner to Dr. Stokes, June 16, 1961

Aims C. McGuinness, M.D.

NEW YORK, N. Y.

I WAS both moved and honored that I had been selected to give an after-dinner talk tonight on this occasion in honor of Dr. Stokes. I was moved because of my personal affection for him and because of my gratitude for the very major role he has played in guiding the direction of my own professional career; I was honored because so many of you in this room are so much better qualified to give this talk than I.

Although time does not permit anything like a comprehensive review of Dr. Stokes's manifold interests and accomplishments, I will try to touch on some of his major contributions.

Dr. Stokes always has had deep concern for constant improvement in the patient care and teaching activities of The Children's Hospital. However, from the very beginning he has developed his program on the conviction that strength in both teaching and patient care rests on the base of a strong program in research. The remarks that follow will, to a major degree, concern the development of research in The Children's Hospital and in the Department of Pediatrics under Dr. Stokes's leadership, and largely those research activities with which Dr. Stokes personally has been associated which have extended his influence to the national and international scene.

Thirty years ago, Dr. Stokes became acting chairman and chief of the service in the absence of Dr. John Claxton Gittings who had gone into temporary retirement. By this time, it is important to point out, Dr. Stokes's reputation in the field of academic pediatrics had also received national recognition for in

May of that year he was one of five elected to membership in the American Pediatric Society. At age 34 he was certainly one of the youngest individuals ever taken into the organization. This is made all the more remarkable because of Dr. Stokes's training and experience during the eleven years which had elapsed since he had received his medical degree from the University of Pennsylvania in 1920, which had included but four months of formal inservice hospital training in pediatrics, a short period here at The Children's Hospital in the spring of 1922. This had been preceded by 20 months in medicine at the Massachusetts General Hospital, and ten months at the Pennsylvania Hospital, the latter service to fulfill the surgical requirements of the Pennsylvania State Board. On the advice of his distinguished physician father, the late Dr. Joseph Stokes of Moorestown, Dr. Stokes spent two years in general practice before limiting his practice to pediatrics and joining the faculty of the University of Pennsylvania as an instructor in pediatrics in 1924. One can be certain that this period in general practice, coupled with six or seven years in a large private pediatric practice, played a strong part in molding his deep understanding of and interest in family problems, a concern to be later manifested in his strong support of the Philadelphia Child Guidance Clinic and its close affiliation with The Children's Hospital.

Within two years Dr. Stokes had made a tremendous impact on the whole departmental program. He was quickly recognized as an outstanding clinician possessed of extraordinary intellectual curiosity, a keen interest in research, and a quiet and delightful charm that from the beginning commanded the affection and loyalty of the entire staff, young and old.

In 1932 Philadelphia suffered a severe epidemic of poliomyelitis, and in collaboration with Drs. Milton Rapoport, Irving Wolman, the late Howard Childs Carpenter and Julius Margolis, Dr. Stokes conducted extensive studies of the prophylactic use of parents' whole blood. During this same year there was published the first report on studies of the antirachitic value of vitamin D milk, studies in which Dr. John McK. Mitchell was the principal investigator with Dr. Dorothy V. Whipple, Dr. Stokes, and the late Dr. John Eiman, as collaborators. Dr. Milton Rapoport and Dr. Whipple were to continue these studies for a number of years.

Dr. Mitchell I. Rubin, now Professor and Chairman of the Department of Pediatrics at the University of Buffalo, came from the Harriet Lane Home to head up the outpatient Department and a newly created medical service, and Dr. Stokes had the nucleus of the full-time staff which was to assist him in putting The Children's Hospital of Philadelphia into the forefront of children's medical centers in the years not very far ahead. Dr. Rubin and Dr. Rapoport soon began their studies of the kidney, the chief area of research interest of both of them today.

Dr. Stokes's personal research interests were now focusing on the field of infection, then still the leading cause of morbidity and mortality in the pediatric age group. Dr. Stuart Mudd and his associates in the Department of Bacteriology at the University, in collaboration with the late Dr. John Reichel at Sharp and Dohme, had by early 1934 brought the lyophile process for vacuum drying serum to the point for practical application. Dr. Stokes promptly recognized the advantages this process would provide in the collection and storage of various kinds of immune sera, particularly human sera, and in collaboration with Dr. Mudd and Dr. Rudolph Roddy he conducted preliminary clinical trials in the spring of 1934. Immediately on the completion of my house officership that July, I was given the opportunity to extend these studies and to develop the work of the Serum Exchange. Although I was nominally to be in charge of its activities for the next 21 years, Dr. Stokes always was, and I am sure still is, the guiding light behind its scientific activities.

In 1934 Dr. Richard E. Shope of the Rockefeller Institute, a close friend of Dr. Stokes, first reported the successful immunization of hogs against swine influenza by

the intramuscular injection of active swine influenza virus. Smith, Andrews, and Laidlaw in England and Francis, Magill, and Shope in this country had at about the same time demonstrated that ferrets and mice could be infected by the virus of both swine and human influenza and could be immunized against the disease. In view of the successful production of active immunity in lower animals and in view of the prevalence of human influenza in such widely separated places as Australia, England, Puerto Rico, New York, Alaska, and Philadelphia, Dr. Stokes felt it was essential to study the production of active immunity in human beings. In the late summer of 1935 he therefore initiated what was to become a series of extremely important and historical studies of influenza including the first field studies of human vaccination against the epidemic disease. His initial collaborators included Dr. Alice D. Chenoweth (Pate), now of the Children's Bureau, the late Arthur D. Waltz, then Pathologist at the Hospital, and Mrs. Dorothy R. Shaw who did the virologic work. Dr. Paul Langner and I participated in the clinical studies beginning in 1936. In 1939 Dr. Werner Henle took over the laboratory aspects of the influenza studies, to be joined shortly by his wife, Dr. Gertrude Henle.

In July, 1939, Dr. Stokes became William Bennet Professor and Chairman of the Department, and Physician-in-Chief to The Children's Hospital. On Sept. 1, 1939, Hitler invaded Poland, and two days later Britain and France declared war on Germany. World War II was on. Mounting interest and concern for problems related to the control of infectious diseases in both military and civilian populations led to the rapid expansion of the work at The Children's Hospital. Dr. Stokes in collaboration with Professor William Wells and his wife, Dr. M. W. Wells, and Dr. Theodore S. Wilder began extensive studies of ultraviolet light in the control of airborne infection. At the same time Dr. T. N. Harris and Dr. Stokes began their studies of air disinfection with propylene glycol vapors. During this same period Dr. Charles F. Church and Mrs. Claire Foster Weaver were actively engaged in their studies of diet and resistance to infection.

It was imperative at this point that Dr. Stokes have some relief from his then, as ever since, almost overwhelming responsibilities. To that end, in 1940, Dr. T. F. McNair Scott transferred from Temple University to the University of Pennsylvania and The Children's Hospital, as Research Professor of Pediatrics and Director of the Research Department. With him came Dr. Alex J. Steigman (now Professor of Child Health at Louisville, Kentucky) as Research Resident. But after ten months they both left to join the American Red Cross Harvard Unit in England. Later army service kept Dr. Scott away from the department until January, 1946, when he resumed his duties.

In 1940 with the work on influenza and airborne infections going along in the hands of capable teams, Dr. Stokes turned his attention to measles, another disease which with its complications, had been a very serious problem in World War I. With the collaboration of the late Dr. Geoffrey Rake, then of the Squibb Institute, Dr. Morris Shaffer, now of Tulane, Dr. Gerald O'Neill, and later Dr. Elizabeth P. Maris, extensive studies on measles vaccination were carried on over a period of 3 or 4 years. These studies ended when the first substantial trial of vaccine proved ineffective in an epidemic two years after the injections, and concurrent difficulties were being experienced in maintenance of the attenuated strain. Dr. John Enders and others now seem to have tamed this virus once more, and after a lapse of about twenty years Dr. Stokes is back at work in the measles field.

On June 14, 1940, the French surrendered to Hitler, and two months later Dr. Stokes undertook a mission to unoccupied France on behalf of the American Friends Service Committee to make a nutrition survey of the area.

When the Secretary of War authorized the establishment of the Board for the Control of Influenza and Other Epidemic Diseases in the Army on Jan. 11, 1941 (now the Armed Forces Epidemiological Board),

it is not surprising that Dr. Stokes was called upon to head up one of its 10 commissions, the Commission on Measles and Mumps, and to be a member of the Commission on Cross Infections in Hospitals, later known as the Commission on Airborne Infections.

In the spring of 1942 in preparation for studies of passive immunization in mumps, a large batch of convalescent serum was collected by the Serum Exchange Staff from recruits at Fort Bragg, North Carolina. Little attention was paid at that time to the fact that curious outbreaks of jaundice had just begun to appear in Army personnel, and several weeks later I generously administered a large dose of this serum to Dr. Francis D. W. Lukens who had come down with a rather severe case of mumps. The mumps was mild as compared with the subsequent serum hepatitis from which Dr. Lukens barely recovered. In any event it was this fortuitous series of events which started Dr. Stokes on his studies of serum and infectious hepatitis, and probably his major scientific contributions during the World War II period, contributions then made principally in collaboration with Drs. Sydney Gellis and John R. Neefe.

The hazard of serum hepatitis dampened further attempts to employ human serum in the prevention and treatment of mumps. However, Dr. John F. Enders had grown the mumps virus in the chick embryo by 1943 and shortly thereafter Dr. Stokes in collaboration with Dr. Enders and with the assistance of Dr. Elizabeth Maris and Dr. L. W. Kane began extensive and successful work with vaccine and in the development of the skin test for susceptibility to the disease. These studies were to be continued over a number of years, Dr. Gertrude Henle assuming responsibility for the virologic work. Drs. Jane and Carroll Burgoon participated in much of the clinical research, and conducted one particularly interesting study in Alaska.

Dr. Stokes may tell you that his chief contribution to the War effort was his part in the establishment of the special allotment of 25 medical officers for the Army Epidemiological Board, accomplished through a personal contact with the then Secretary of War, the late Mr. Henry L. Stimson.

The United States was now in the War, and the year 1942 was a difficult one for the members of the several commissions who were still of military age. Although the Army Surgeon General at first hoped that members of the Epidemiological Board Commission who chose to enter the Army could be retained in their research positions for the Board, it seemed by the fall of 1942 that this was not going to be accomplished through normal channels. Dr. Horace Hodes and the late Dr. Francis Schwentker of Johns Hopkins were two of several commission members who already had resigned to join the Navy, and several others of us planned to do likewise. Dr. Stokes rightly considered the situation both serious and ridiculous. Not being one impressed either with brass or red tape, he got straight to Secretary Stimson, through mutual friends, and the situation was almost immediately in hand. On Dec. 15, 1942, a special allotment of 25 officers was established for the Board, and within 24 hours I had been inducted as its first recruit. Albert Sabin was the second, and then came people like John Dingle, Sydney Gellis, John Neefe, Alexander Langmuir, Clayton Loosli, and a number of others with whose names you would be familiar.

I was subsequently to become Assistant Administrator of the Board under General Stanhope Bayne-Jones and, if Dr. Stokes thinks his chief contribution was the securing of a special allotment of officers, my contribution as one of those officers was to endeavor to keep Joe Stokes and military red tape at a reasonably safe distance from one another.

I think a nice expression concerning a portion of Dr. Stokes's contribution to the national effort during the years of World War II may be found in the following citation for the Medal of Freedom which, by direction of the President, he was awarded on May 6, 1946:

"Dr. Joseph Stokes, Jr., Consultant to the Secretary of War and Director of the Com-

mission on Measles and Mumps, Army Epidemiological Board, rendered meritorious service in the investigation and control of infectious hepatitis among troops in the Mediterranean and European Theaters of Operations during the period from 17 October to 23 December 1944. Carrying into the field of military campaign in Italy the knowledge, discoveries and experience he had gained from the study of hepatitis in the United States he participated in the use of immune serum globulin for the prophylactic immunization of troops exposed to this disease at a time when infectious hepatitis had assumed great importance as a hazard to health and an impediment to military operations. By his scientific investigations and their application in the field in communities and institutions Dr. Stokes has conferred benefits upon both military and civilian public health."

How Dr. Stokes and his staff, including such stalwarts as John Porter Scott, Milton Rapoport, Jim Reilly, Henry Lee, Harriet Felton, Rachel Ash, and Julian Lyon, managed to get through the War years and still retain a semblance of sanity is difficult to understand, but they did and with a magnificent record of accomplishment. It was, incidentally, during this period that Dr. Seymour S. Cohen joined the research staff and began his distinguished work on biochemistry of viruses which has added so much luster to the Department. For a number of years he was to be assisted in this work by Dr. Dwight B. McNair Scott. In 1944 Dr. Paul Gyorgy joined the Department to continue his own research on nutrition and to have responsibility for the clinical service on Ward G at the University Hospital.

At the conclusion of the War, many of us returned to resume our old jobs and to take on additional responsibilities as well. In the next few years many new names were added. Many of these came from the ranks of those who had served as residents in the immediate postwar period. Among these new additions were: Andy Hunt, Wallace McCrory, Al Bongiovanni, Sidney Friedman, Bob Kaye, Lew Coriell, Giulio Barbero, Harold Lecks, Lew Barness, John Farquhar, Mike Sigel, and Tom Boggs. Each of these subsequently has became a research investigator in his own right.

A milestone in the history of the Hospital was the institution of the full-time Department of Surgery under Dr. C. Everett Koop in 1949, a department to be strengthened by its subsequent broadening to include neurosurgery under Gene Spitz and urology under Alex Michie. The establishment of a full-time Department of Radiology under John Hope in 1951 greatly reinforced both clinical investigation and clinical service.

Dr. Stokes's interest in hepatitis has remained active over the years, and many of you are more familiar than I with the work conducted in collaboration with the Henles, and with the assistance of such people as John Farquhar, Frank Shaffer, Miles Drake, and others.

The potential of the use of human antibodies, particularly in their gamma globulin form, also continues to be substantial. In collaboration with Joseph Smolens and with the assistance of Elizabeth McGee and Virginia Hunter, Dr. Stokes has conducted extensive studies of plasmapheresis and of plasma sterilization.

One not close to Dr. Stokes might be unaware of the extent to which his wisdom and guidance have been depended upon by the American Friends Service Committee. Reference was made earlier to his mission to unoccupied France in 1940. In August and September, 1959, he was chief of a mission to the Soviet Union which served as the first phase of a cultural exchange program being undertaken by the Soviets and the American Friends Service Committee. Many of you have been so fortunate as to have heard his saga of this trip.

I think it is of interest that Dr. Stokes's initial major project in the field of infectious diseases concerned passive immunization against poliomyelitis. Just over a quarter of a century later he is engaged in studies of active immunization against poliomyelitis with live virus in collaboration with Dr.

Hilary Koprowski and their respective associates.

As I conclude these comments on one phase of Dr. Stokes's productive life I am again mindful of how much I have left out. I have been unable to give any kind of real expression to the influence he has had on each of us who has been so fortunate as to be one of his associates. I use the word "associate" for Joe never seems to act toward anyone as though that person were an assistant. We always have been part of an exciting team of which he is the leader, and always bound on an equally exciting venture. I have been unable to elaborate on such things as Dr. Stokes and the plans for the future of The Children's Hospital. Nor have I had an opportunity to give more than passing attention to Dr. Stokes and American Pediatrics. Those of you who attend the presentation of the Howland Medal and Award at the next annual meeting of the American Pediatric Society will hear more about the part Dr. Stokes has played in the evolution of pediatrics in this country and around the world. He will be the eleventh recipient of this award which was established in 1952 "to honor those who by their contributions to pediatrics have aided in its development." That, incidentally, is part of the original statement of purpose of the American Pediatric Society.

Finally, it is appropriate to point out that through his mother and father Joe Stokes inherited a great tradition of service to humanity and the community. In spite of this heritage, however, he would have had a difficult time in accomplishing his goals without the strong and devoted support of Frances Elkington Stokes and their four children.

I join with all his friends in congratulating Joe on his remarkable career; in expressing the gratitude of all of us who have been privileged to serve with him; and in extending our best wishes for happiness and success in the next phase of his activities. I gather, incidentally, this may consist of working for The Children's Hospital of Philadelphia and the Department of Pediatrics even harder than he had in the past—if that is conceivably possible.

Concerning Dr. Joseph Stokes, Jr.

Isaac Starr, M.D.

PHILADELPHIA, PA.

WHEN a number of an important medical journal is dedicated to a man worthy of this honor it seems appropriate to begin with a brief account of the man himself, his background, and his accomplishments, for the benefit of those readers who may not know him personally.

Ever since the American Revolution, except for a period of six years, there has been a Dr. Stokes practicing in the little town of Moorestown, New Jersey, now almost a suburb of Philadelphia, though on the Jersey side of the Delaware River. Dr. Joseph Stokes, Senior, practiced there from 1883 to 1943, and he was no man to be passed over lightly. He is remembered not only because he was a respected and well-loved physician and a pillar of the community, but also for a most unusual accomplishment. At the Medical School of the University of Pennsylvania which he attended from 1880 to 1883, he received a mark of 100 in every examination, a record never equaled before or since.

Joseph Stokes, Jr., was the second son of this prodigy to become a doctor and following his family's Quaker traditions he attended first the Moorestown Friends School and then Haverford College.

Perhaps the event in Joe's college career which most of us remember best is a trip to England taken by the Haverford Cricket Team, both team and trip unusual in the American College scene. One gathers that the more experienced English school boys usually defeated the Americans. But Joe was a fast bowler and there were times when the pitch was right and the English wickets flew in all directions. One of these occasions was recalled by Dr. Bernard Schlesinger of The Hospital for Sick Children, Great Ormond Street, London, when he came to this country to take part in the celebration of the 100th Annversary of the founding of The Children's Hospital of Philadelphia. An old Uppingham boy himself and present at the game mentioned, Dr. Schlesinger produced a copy of the school paper which noted: "In the match with Uppingham School (June 30, 1914) Haverford defeated the home team by 244 runs to 80. J. Stokes was one of the towers of strength for his side. He bowled practically unchanged throughout and had the remarkable analysis of 7 wickets for 27 runs." And one gathers that much the same thing happened at a later match at Eton.

Medical School at the University of Pennsylvania where I was a classmate, and Internship at the Massachusetts General Hospital, where I found myself immediately behind him on the West Medical Service, completed the more formal part of Joe's education.

After a brief interval of general practice in Germantown, he turned to pediatrics and at the age of 35 years he was asked to become "acting" chief physician to The Children's Hospital of Philadelphia. Obviously

the authorities feared to give the full responsibility to so young a man. In 1939 he became "chief physician" without qualification of the oldest children's hospital in the United States and the second oldest in the English-speaking world, only the hospital on Great Ormond Street in London being older. In 1939 he also became Professor of Pediatrics at the Medical School of the University of Pennsylvania and has been chairman of this department since that time.

Throughout his long medical career, Joe has always exhibited great talents as an organizer and administrator and his accomplishments can best be described under several heads. His research interest has always been in the field of the infectious diseases, especially infectious hepatitis, and he has concerned himself with organizing studies of their epidemiology, and for the purpose of determining the effectiveness of vaccines, sera, gamma globulin, and other therapeutic agents which might be used against them, for both therapy and prophylaxis. The method of attack was, like all his thinking, simple and direct.

During the war, infectious hepatitis became a serious military problem, and, as the disease could not be transmitted to animals, animal experiments provided no help. Progress against the problem lagged until Joe, doubtless aided by his lifelong connection with the Society of Friends, secured the service of large numbers of conscientious objectors who, not lacking in courage, and aiming to make their contributions in other ways than by fighting, volunteered for inoculation experiments which sought to discover how infectious hepatitis was transferred, and how such transference could be prevented. The results greatly increased our knowledge of this disease. And, when this source of human subjects declined with the end of the war, Joe greatly advanced work on other infections by finding volunteers in New Jersey State Institutions. Indeed, he is reliably reported to have run experiments on mumps on his first grandson. There is some doubt whether an infant of 6 months could be properly called a volunteer, but there is no doubt that, after inhaling a live virus, the child's antibodies rose. Surely, Peter Whitson Stokes, who began his career in investigative work at so early an age, bids fair to uphold well the distinguished medical tradition of the Stokes family.

Joe's extensive wartime service had two other highlights, the first so characteristic of his ability to get important things done despite all obstacles. Finding a vital part of the Army Epidemiological Board's program in jeopardy because Selective Service was drafting all the Board's trained personnel for routine medical work, and unable to get action through the usual channels of command, Joe took advantage of a slight personal contact with the Secretary of War to present his case directly at the top, and with complete success.

Later, much to the amusement of friends with knowledge of his long Quaker background, because of an alarming epidemic of infectious hepatitis in Air Force personnel in Italy, Joe suddenly found himself in uniform—very necessary because, had his plane been brought down behind enemy lines, he might otherwise have been shot as a spy—and with a rank equivalent to that of colonel. The gamma globulin he brought to Italy stopped the epidemic and soon he found himself awarded the Medal of Freedom by the United States War Department. We have *not* seen him wearing it on his uniform!

Success with human gamma globulin in the prevention of hepatitis led to steps to secure more of this effective, but expensive, product. Following a lead set by Dr. Edwin Cohn and his colleagues, Joe organized an effort which turned the Cohn techniques of separating serum from plasma to practical utility. This work showed that, when the red cells are returned to a blood donor, he can be bled as often as once a week without harm; and so each donor became capable of supplying a greatly increased amount of the blood serum from which the precious gamma globulin can be readily separated.

As is always the case when new ideas and new energy are brought into a medical insti-

tution, both bright young men and new funds were soon attracted to the old Children's Hospital. Now well known to those in Washington for his help during the war, government support of Joe's efforts continued during the peaceful years which followed. Such funds, and those of other interested donors, provided increased facilities. Joe became known as a good man to work under, so new talent was attracted and gradually a new spirit pervaded the whole staff. The magnitude of this accomplishment becomes evident when one realizes that, when Joe became chief medical officer, morale was low, research was pretty much at a standstill, and the funds available for its support were practically nil. Last year, in 1959-1960, 74 papers were published by the staff of the Hospital and the budgets available for research support totaled $1,150,000. But these figures, impressive as they are, testify to only one aspect of the growth of a spirit of alertness and scientific interest which resulted in steady improvement in the care of the sick children admitted to the hospital, and to increased preventive services for the community.

Finally, one cannot close without a tribute to Joe's humanitarian interest, and to the efforts he has directed toward alleviating the nutritional problems of depressed areas. These concerns led him, under the auspices of the Friends Service Committee, or of the World Health Organization, to Occupied France during the recent war, and later to Jamaica and Guatemala. He has been an adviser to two White House Conferences on Children and Youth, and was appointed by the President to the planning committee of the more recent one. Also, he was one of the three founders of the most successful Marriage Council of Philadelphia, and the founder of the first nursery school in the city.

He has received an honorary degree from his old college, Haverford, and was also honored by a citation at the celebration of the 150th Anniversary of the Massachusetts General Hospital. He has also received the Chapin Medal from the City of Providence, the Wilson Medal of the Clinical and Climatological Society, and the Shaffrey Medal from St. Joseph's College. He was elected to membership in the American Philosophical Society in 1953; he is a member of the Committee of Rehabilitation of the United States State Department and was one of the founders of the Society for Pediatric Research. He has been president both of this society and of the American Pediatric Society.

There is no way of accurately judging the relative merits of the many excellent departments of pediatrics now in the United States, but I must close by testifying that a number of distinguished European pediatricians, who recently traveled widely in North America, returned to their countries convinced that one of the greatest, if not the greatest, was at the University of Pennsylvania.

Tribute to Dr. Stokes

Edwards A. Park, M.D.

BALTIMORE, MD.

TO ME he seems at the height of his powers. It will be too bad if he is now unable to carry out his original designs for the new Children's Hospital, planned peculiarly with the happiness and general welfare of the children in mind as well as their medical needs. The towering children's hospitals of today are too much in the nature of factories for the production of medical care, and with their glittering glass and immaculate rooms in danger of seeming to children, suddenly snatched from their homes, a kind of prison. The problem of constructing a hospital safe for infants has never been solved, and the planning of a hospital for older children is far more complicated than for adults.

Joe is not a Quaker for nothing. He possesses in high degree the social consciousness, detachment, and purity of motive which have won for the Friends a universal confidence and given them, by general consent, a license of freedom of action between warring forces which is unique in history. They are the true peacemakers, and the meek of the Beatitudes, and what a relief at the present time, if they could inherit the earth! But Joe has also in high degree the Quaker practicality. I have watched him in command of his department over the years with unqualified admiration. I never knew anyone so watchful of progress in the fundamental sciences, so quick to perceive application of new ideas and methods to the problems of children and so expeditious in getting the work under way. Not so long ago it was not easy to finance new investigations. Then it was necessary to find funds from private sources, but, such was the confidence which Joe's character and judgment inspired, the necessary support seemed to come for the mere asking.

It would be a mistake, however, to think that Joe's interests have been confined to the immediately practical, for the opposite is true. He has grouped around him a set of able investigators selected because of their abilities in special fields in the fundamental sciences where Joe thought new knowledge, pertinent to the welfare of children, could be obtained. Moreover, once started on their programs, he exercised the too rare virtue of leaving them alone to develop and carry out their ideas. Indeed I do not know of any children's clinic in which so large a share of the intellectual activity is concentrated on fundamental inquiries.

Joe's activities have not remained confined to his department but have extended far into the world outside, causing him to travel widely to distant countries to investigate and to organize relief and these missions of Quaker origin he has carried out with the quick intellectual grasp, detachment, and

common sense characteristic of his make-up. His wealth of experience derived from these travels makes him a most interesting companion.

I could write much more about Joe but summarize by saying that he is one of the most splendid and altogether admirable characters I have known in my 83 years, a leader of great ability and one of the finest personal influences medicine in this country has known.

Expressions of esteem from Dr. Stokes's friends and colleagues

Aspects of his life, personality, and contributions as reflected in extracts from letters written to and about him on the occasion of his retirement

Waldo E. Nelson, M.D., Philadelphia, Pa.

These years have brought ever-increasing evidence of your leadership and of its effects upon the growth of pediatrics, not only in Philadelphia but nationally and internationally as well. It has been a privilege to be here in Philadelphia with you, and I would want to add that you have done much to aid our efforts in the northern part of the city.

Herbert C. Miller, M.D., Kansas City, Kan.

It is particularly significant that The Children's Hospital of Philadelphia, the oldest in the country, has under his leadership set an enviable example of community support for pediatric education and research, the benefits from which have extended far beyond the confines of Philadelphia. The affiliation of independent children's hospitals with universities has been of inestimable help to pediatric education.

Professor Alan Moncrieff, London, England

The present exchange of residents between your hospital and ours is a symbol of a professional friendship renewed and enhanced by your centenary celebrations in 1955. We believe it was our first hospital in Great Ormond Street that stimulated your founders and we are proud of our kid sister.

Philip S. Barba, M.D., Philadelphia, Pa.

The development of the Department of Pediatrics as a separate unit from Medicine resulted directly from your efforts, from your courage and firmness in pushing the issue, and your ability to prove the desirability of that procedure. I remember this was not done without stirring up Newton's third law of motion and some of the reactions were far from cool.

Harold K. Faber, M.D., San Francisco, Calif.

One memory comes back to me of an occasion when Joe himself was not present—my first visit to The Children's Hospital of Philadelphia and my provincial astonishment at the superb and many-faceted establishment comprising clinical material, teaching, and research there which had developed under his guiding and productive hand.

Prof. S. van Creveld, Amsterdam, Netherlands

In this clinic and in this research department fundamentally scientific research and work with a more direct and practical application went hand in hand.

J. Harry Ebbs, M.D., Toronto, Ont.

I shall always remember and be grateful for your contribution and valuable help to me in planning the scientific program for the Ninth International Congress of Pediatrics.

S. Z. Levine, M.D., New York, N. Y.

It has not been my privilege to work with you as a faculty colleague but we have joined

together as comembers of committees, as coeditors of pediatric journals, and as colleagues in many undertakings over the years. Your wide interests and broad comprehension, your wisdom and tolerance, and a trained and systematic mind have been reflected in sound judgments and thoughtful decisions in all our deliberations. But even more impressive has been the reaction of your colleagues to your contributions always delivered with a graceful turn of the phrase. Personal dignity, courtesy and friendliness, a gentle firmness, becoming modesty, and a moral integrity have served to eliminate rancor from discussions and time and again have won opponents to your side.

Grover Powers, M.D., New Haven, Conn.

But seriously, you have been a leader in pediatrics in Philadelphia, America, and abroad so long that professional lives cannot be the same with you out of active practice.

Francis Scott Smyth, M.D., San Francisco, Calif.

It must give you deep satisfaction to look back on the number of citizens whom you treated as children and to the students so many of whom have brought credit to themselves and their teacher, and to scientific reputation established by the institution which you have so ably led!

A. Ashley Weech, M.D., Cincinnati, Ohio

Indelibly inscribed to your credit are many years of helpful deeds and wise jurisdiction in a world-famous department of pediatrics. You have earned the right to meditate upon them all with inner satisfaction and to glory in the knowledge of what you have accomplished.

Prof. J. H. P. Jonxis, Groningen, Netherlands

I was happy to have contact with you and to learn from your great knowledge of pediatrics.

Charles C. Chapple, M.D., Washington, D. C.

If there were to be just one quality chosen as his most characteristic characteristic it would be his steadfastness, and I mean that word in its finest sense.

Professor Robert Debré, Paris, France

My meetings with Joseph Stokes have always been of great charm. I cannot use another term. During each of my visits to the United States I have derived much pleasure and great benefit from meeting the best pediatricians of the New World. With Joseph Stokes I have every time experienced the feeling that I have just expressed.

Rustin McIntosh, M.D., New York, N. Y.

You have done many kind things, Joe, in the course of a busy life—probably none kinder than your response to my plea, during the blackest of the war years, that you come to New York in order to tell the Babies Hospital Alumni Association something of the impact of the Nazi invasion on life in France as you had seen it in the course of your travels with the relief mission. All of us recall with gratitude your vivid account, and I in particular retain an everlasting appreciation of your willingness to share with us your experiences and give us the benefit of your time during those days when we were all hard pressed and no one had time to spare. This is an episode which I enjoy reminding you of because its recollection has given me so much pleasure in the course of the twenty years or so since it occurred.

Professor G. M. H. Veeneklaas, Leiden, Netherlands

The Quakers have a tender spot in many European hearts. They were the first on the spot to provide immediate help after the end of World War II without asking any personal comfort. We know that you were one of these.

Do you remember that I asked you what you did with your feelings of aggression and hostility? And your answer that you identified with the football players on the T.V.?

John D. Bridgers, M.D., Philadelphia, Pa.

There are few people who could withstand the stresses of attending to the multitudinous tasks and duties accepted by Dr. Stokes with the evenness of temperament which he displays. Many acquaintances have been impressed by this and have wondered what occult devices he has for "letting off steam" and discharging his hostilities. The people

who ask this question, however, have never been passengers in an automobile which he was driving. He is truly the "Barney Oldfield of Pediatrics." His ability to move in unpredictable ways, through complicated traffic, and have things work out satisfactorily is undoubtedly akin to his ability to weld together all sorts of unlikely alliances and collaborations into successful projects.

The house staff slowly learned that he seemed to use the acuity given by his fenestration when it suited him, but at times retreated into his preoperative status of not being able to hear when he was distracted with other problems. It led one of the house staff to observe that this was the first fenestration procedure on record with a built-in volume control. Those close to Dr. Stokes have learned that he keeps "his ear to the ground" much more often than one would suspect, and we have learned this when summoned for a "man-to-man" chat.

This true sense of humor—this introspective ability to laugh at his own foibles—is undoubtedly at the base of his amazing retention of youthful physiologic, psychologic, and intellectual capacities. Those of us who call him friend and mentor know that as he looks into the sunset of one career he is actually searching for new hills to climb.

Charles A. Janeway, M.D., Boston, Mass.

I look forward to your liberation from the cares of administrative office, because then your extraordinary energy and dedicated imagination will be unleashed to meet some of the myriad problems of applied microbiology and human relations which need your help in their solution.

Dr. Frederico Gomez, Mexico City, Mexico

We hope that even if you retire from The Children's Hospital of Philadelphia, you continue lending your services to continental pediatrics in other fields where your valuable experience may serve to encourage the progress of science for the benefit of childhood in North, Central, and South America.

Prof. Arvo Ylppö, Helsinki, Finland

Like a young scout you helped us to make a fire for the coffee pot on our island when we made the motorboat trip to my fishing base in the open Baltic Sea. And then the Finnish Sauna (bath), and the trouble in writing your name in my guestbook, i.e, the wall of the stove in our summer house. As a pediatrician you pointed out that this was the kind of guestbook children cannot tear, and that is true. Your artistically written name on the stove is still before my eyes, reminding me of you and of other good friends from many countries who have visited us since 1926 when the first names were written.

Prof. N. I. Krasnogorski, Leningrad, U.S.S.R.

Our family congratulates you on the occasion of your sixty-fifth birthday and hopes that your health will continue to be excellent. With warm regards.

The suppression of transferred lymph node cells in adult and neonatal rabbits

T. N. Harris, M.D.,* and Susanna Harris, Ph.D.

PHILADELPHIA, PA.

IN THE search for the source of antibodies, which has concerned immunologists for over half a century, attention was focused fairly early on the lymphatic system. Special interest in this organ system followed the direct demonstration by McMaster and his colleagues[1, 2] of antibody in extracts of lymph nodes draining the sites of injection of antigens, under appropriate circumstances. The lymphatic system is, however, widespread throughout the mammalian body, and its components, the lymph nodes and spleen, are complex organs containing a variety of cell types. More detailed analysis of this problem has, in recent years, involved the use of a technique which had been touched upon by early workers.[3, 4] This technique, the transfer of immunologically competent cells from one animal to another of the same species, was brought into extensive use following the work of Landsteiner and Chase[5] on the transfer of delayed hypersensitivity. In the course of later studies Chase[6] transferred lymph node cells from guinea pigs which had not only been sensitized with a simple chemical compound (picryl chloride) but also injected with sheep erythrocytes. The recipients of these cells developed hypersensitivity to the picryl chloride and anaphylactogenic antibody to this antigen and hemolysin to the erythrocytes. This demonstration of the transfer of antibody formation by lymph node cells was applied in many laboratories with a number of species (rabbit, guinea pig, mouse) and a variety of antigens (*Shigella paradysenteriae, Eberthella typhosa*, erythrocytes, diphtheria toxoid, and bovine serum albumin and globulin).[7-10]

The transfer of lymph node cells from antigen-injected donor rabbits. In this laboratory, early experiments were performed with Shigella, sheep erythrocytes, and influenza virus. At an appropriate time after injection of antigen into the foot pad of the rabbit the draining (popliteal) lymph node was removed and the cells obtained from that node were washed and transferred to fresh recipient rabbits. On the day following cell transfer the sera of the recipient animals were found to contain homologous antibody. The level of antibody increased until the third or fourth day, and began to decline

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after the seventh day.[11] This curve of antibody concentration versus time, as well as other experimental observations, indicated that the antibody found in the recipient's serum was not carried over in the cell suspension, and was not due to antibody formation by the recipient's tissues in response to antigenic material carried over in the cell suspension. Rather, the data obtained, especially the finding that only viable cells were effective, strongly suggested that the antibody found in the recipient was due to a continued function of antibody formation by the transferred cells in the new host.

In experiments with Shigella it had been found that cells removed 4 days or 3 days after the injection of antigen into the donors gave rise to the appearance of antibody in recipients on the first day after transfer. With further reduction of the interval between the injection of antigen into the donor and the collection of its lymph nodes it was found that antibody appeared in the recipient animal later with respect to the time of cell transfer. With an interval of 2 days, antibody appeared in the recipient's serum on the second day after cell transfer; and with an interval of 1 day, antibody appeared on the third day after transfer.[12] Further, it was found that with shorter intervals in the donor animal more antigen was carried over with the cells. To prevent active immunization of the recipient by such antigenic material, it was necessary to use deep x-irradiation of the recipients before cell transfer. The irradiation of the recipients was found not to interfere with the effect of the cell transfer. On the contrary, it enhanced the level of antibody which appeared in the recipient.[13]

The transfer of lymph node cells incubated in vitro with Shigella antigen. Further experiments led to the observation that lymph node cells from donor rabbits which had not been injected with the antigen could be incubated in vitro with Shigella or with soluble forms of the antigen (agglutinogen) of this organism and transferred to recipient rabbits with the subsequent appearance of agglutinin in the sera of the latter.[14] In this case the antibody appeared on the fourth day after cell transfer, and thereafter followed the same curve of concentration versus time as had been found in the case of cells transferred from donor animals injected with Shigella. Fig. 1 shows the uniformity of the time of appearance of antibody in relation to the time of contact between the cells to be transferred and the antigen, regardless of the time of collection of lymph node cells from the donor animal.

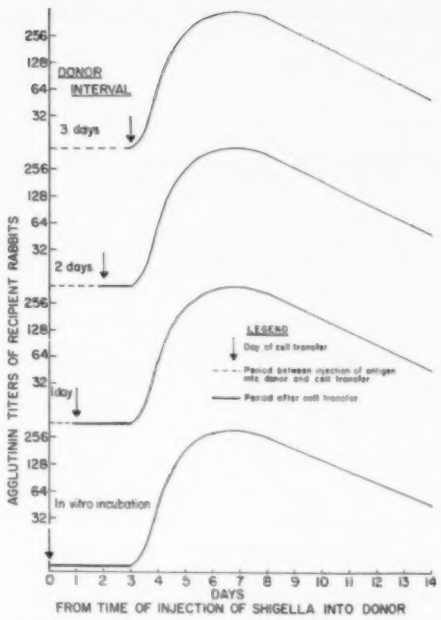

Fig. 1. Diagram of time of appearance of agglutinins to Shigella in the sera of recipient animals, relative to the time of contact between Shigella antigen and the lymph node cells to be transferred. Cells transferred from donor rabbits previously injected with Shigella, or incubated in vitro with soluble antigen derived from Shigella, as indicated. (From Harris, T. N., and Harris, S., *in* Wolstenholme, G. E. W., and O'Connor, M., editors: A Ciba Foundation Symposium on Cellular Aspects of Immunity, 1960, J. & A. Churchill, Ltd.)

Effect of prior injection of donors' leukocytes into recipient animals. From the early experiments in this system it was considered probable that the antibody which appeared in the recipients' sera was synthesized in the transferred lymph node cells, although this was not proved. A further approach to this

problem was made by specifically altering the host tissue environment, and observing the effect of this change on the transferred lymph node cells. This experimental approach arose as a result of the observation by Medawar,[15] and then by many other workers in the field of tissue transplantation, that if a skin graft of a given donor animal is placed on a recipient not genetically identical with the donor, the graft will be rejected, and that if a second graft is placed on the recipient animal from the same donor, this will be rejected more quickly than the first, presumably because of an immunologic reaction of the tissues of the recipient to cell antigens of the donor. Since, in our experiments, the rabbits represent a genetically heterogeneous population, the question arose of whether the recipients of transferred lymph node cells would react to cell antigens of the transferred cells in such a way as would affect the function of those cells.

In order to examine this question experiments were designed so that rabbits which were prospective recipients of lymph node cells were injected with leukocytes obtained from the blood of the prospective donors of the lymph node cells. One week after the leukocyte injection, lymph node cells were obtained from the donors, incubated in vitro with a soluble form of the Shigella antigen, and transferred to the leukocyte-injected rabbits. It was found that antibody did not appear at all in those preinjected with 10 million or more leukocytes, or appeared in very low titer when 1 million leukocytes had been injected, whereas control recipients developed agglutinin titers in the usual range.[16] A diagrammatic representation is shown in Fig. 2 of typical antibody curves observed after transfer of lymph node cells to recipients preinjected with 10^7, 10^6, and 10^5 leukocytes, respectively, as well as control recipients.[20]

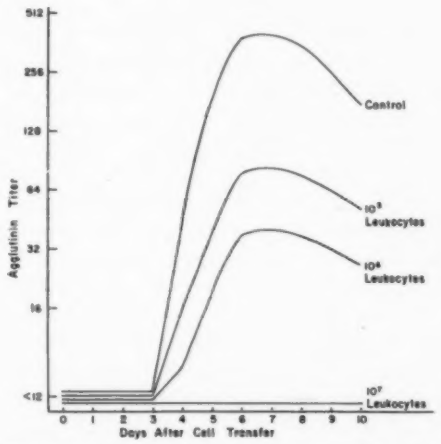

Fig. 2. Diagrammatic curves of antibody levels in recipient rabbits after transfer of lymph node cells incubated in vitro with Shigella-trypsin filtrate. The curves show the effect on antibody production by the transferred cells of the prior injection of different numbers of donors' leukocytes.

Clearly, the prior injection of a sufficient number of donors' leukocytes had altered the environment in the recipients' tissues so as to make it incompatible with the continued functioning of transferred lymph node cells. Thus, whereas in skin homografting the placing of a first skin graft led to an accelerated rejection of the second homograft, in the parallel case of the lymph node cells the injection of leukocytes could cause suppression of the appearance of antibody altogether or, in the case of a partial effect, cause the appearance of the antibody in a lower concentration in the recipient's serum.

The suppression of antibody production by the transferred lymph node cells as shown in Fig. 2 was evident one week after the injection of leukocytes. The effect of variations of this interval was examined. It was found that when 1 day elapsed between the prior injection of leukocytes and transfer of lymph node cells, subsequent titers of the recipients were unaffected, resembling those of the control recipients. Some suppression of the transferred lymph node cells was evident in the case of a 2 day interval, and more suppression with a 3 day interval. At intervals of 4 to 6 days suppression was almost complete. When intervals longer than 1 week were used it was seen that complete suppression could be observed up to an interval of 20 days. With still longer intervals, the maximal level of agglutinin after cell transfer began

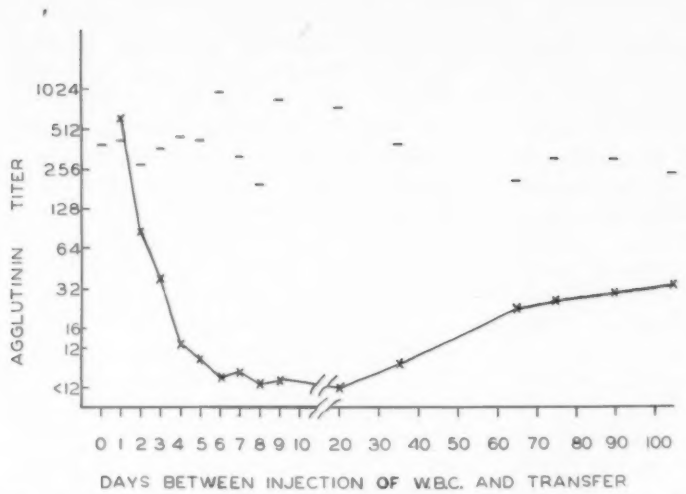

Fig. 3. Geometric mean peak agglutinin titers of recipient rabbits that had been preinjected with leukocytes of other (donor) rabbits at various intervals prior to the transfer of antigen-incubated lymph node cells from the same donor rabbits. Geometric mean peak titers of control (nonpreinjected) rabbits for each group are shown by the horizontal lines. (From Harris, T. N., and Harris, S.: Ann. New York Acad. Sc. **73:** 789, 1958.)

to increase, beginning to approach control levels of agglutinin titers only after 107 days.[16] These findings are shown in Fig. 3.

Suppression of transferred lymph node cells by anti–rabbit-leukocyte antibody. Because of several additional indications that this suppression of antibody synthesis by transferred lymph node cells was immunologic in nature, a search was made for humoral antibodies which could mediate the suppressive effect. Groups of rabbits were injected with leukocytes pooled from many donors. After a given interval the injected rabbits were bled and their sera pooled. The sera were then either injected into recipient rabbits just before transfer of antigen-incubated lymph node cells, or were incubated with such cells before the transfer to recipients. It was found that exposure to such sera suppressed the appearance of antibody after transfer, reproducing the picture of actively induced suppression brought about by the injection of leukocytes into the recipients.[17] Fig. 4 shows the range of the mean maximal titers of recipients in relation to the volume of antileukocyte serum employed, for two kinds of serum pools: those obtained from rabbits given a single injection of pooled rabbit leukocytes, and those from rabbits given 2 such injections, with an interval of 1 month between them. For either type of serum pool, the larger the amount of serum employed, the lower the mean maximal titers found in the recipients. It can be seen that the sera obtained after two injections of leukocytes are more effective than those obtained after a single injection. Thus, for a suppression of maximal titer to a mean value of 32 (corresponding to a reduction to approximately 15 per cent of the control values) the range of amounts required, in the case of antileukocyte serum obtained after one injection, was from 0.8 ml. to 7.9 ml., whereas antileukocyte sera obtained after a second injection showed a similar degree of suppression in amounts of 0.07 ml. to 0.8 ml. This indicated a ratio of about tenfold in the concentration of the suppressive effect in the two types of serum.[18]

Immunologic reactions of neonatal rabbits to antigens of rabbit leukocytes. During the period of the studies just described, consid-

erable interest had grown in the question of the immunologic immaturity of the newborn of various animal species. For a number of reasons neonatal rabbits were used as recipients of lymph node cells from adult rabbit donors. In the course of these experiments it was found that neonatal rabbits could be given antigen-incubated lymph node cells, with the subsequent appearance of antibodies in their sera in at least as high a titer as that found in older recipients, although the total amount of antibody produced per million transferred cells was less. It was also found that the newborn rabbits showed evidence of immunologic reaction to the cell antigens of the transferred cells. Employing the type of experiment described for the older recipients, it was seen that rabbits could react to the injection of donor leukocytes, even when these were given on the day of birth, by suppressing antibody formation by lymph node cells subsequently transferred to them. The reaction was apparently as vigorous in the young rabbits as in the mature recipients, since no difference was observed between the two groups in their reaction to antigens of injected leukocytes at several levels of numbers of leukocytes injected.[19]

This observation was of especial interest in view of the well-known inability of neonatal animals to respond with the formation of detectable amounts of antibody following the injection of a variety of antigens, and in the light of our finding of the role of a serum antibody in the suppression of transferred lymph node cells in older rabbits. Accordingly, a search was undertaken for antileukocyte antibodies in the sera of newborn rabbits. In a series of experiments, young rabbits (from a few days to 4 weeks of age) were injected with leukocytes pooled from the blood of adult rabbits (the same procedure as was used to produce anti–rabbit-leukocyte serum in older rabbits) and then bled at given intervals thereafter. When these sera were tested for their suppressive effect on lymph node cells incubated in vitro with Shigella antigen, they were found to be relatively ineffective. Some of the comparisons of sera produced in older and younger rabbits are shown in Fig. 5. Across the entire graph the horizontal midline represents the control geometric mean maximal titer, used as a base line. Each serum was tested at various volumes from 10 ml. to 1 ml. The geometric mean maximal titers of recipients were compared in each case to the control value for that experiment and the difference is shown, accordingly, above or below the control level (differences given in $\log_2$ steps). On the right side of the graph are shown the results for adult rabbit sera, which, when used in 10 ml. amounts, led to the appearance of maximal recipient titers which were more than 3 $\log_2$ units below the control level. The degree of suppression decreased with decreasing volumes of serum, as noted

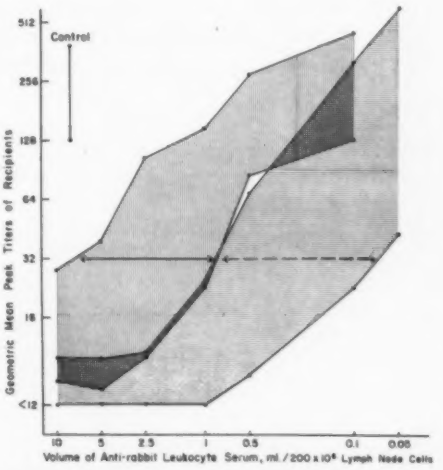

Fig. 4. Comparison of suppressive effect of rabbit anti–rabbit-leukocyte sera obtained after a single injection or 2 injections of rabbit leukocytes. The range of mean maximal recipient titers observed after transfer of antigen-incubated lymph node cells incubated with decreasing volumes of each type of serum, from 10 ml. to 0.1 ml., are shown. The area blocked in by the vertical lines shows the range for sera obtained after a single injection and the area in horizontal lines the range for sera obtained after 2 injections. For each kind of serum the arrow heads give the range of amounts required to produce approximately 85 per cent suppression, i.e., a maximal titer of 32. In the upper left corner of the graph is shown the range of titers of control recipients, injected with antigen-incubated lymph node cells alone.

Table I. Transfer of lymph node cell suppressing effect by cells from lymph nodes draining sites of injection of donor leukocytes*

Experiment No.	*Source of cells obtained from leukocyte-injected rabbits*				
		Neonatal rabbits		*Older rabbits*	
	None (control) mean titer, $\log_2$	*Popliteal lymph node mean titer, $\log_2$*	*Spleen mean titer, $\log_2$*	*Popliteal lymph node mean titer, $\log_2$*	*Spleen mean titer, $\log_2$*
1	9.3	6.0	7.8	6.2	8.4
2	8.1	4.2	7.3	5.5	6.5
3	8.1	4.9	6.8	7.7	7.8

*Comparison of geometric mean maximal anti-Shigella titers of recipients of usual (Shigella antigen–incubated) lymph node cells (controls) with those given, in addition, cells from leukocyte-injected rabbits.

above. In the case of sera produced in the neonatal rabbits (left side of Fig. 5) there is no markedly consistent suppression. There was occasional evidence of suppression when 250×10^6 leukocytes were used for injection instead of the usual 50×10^6, and then only at the 10 ml. level.

Because of the contrast between the ease of active induction of suppression of transferred lymph node cells by the injection of leukocytes into the neonatal rabbits and the failure of anti–rabbit-leukocyte serum produced in young rabbits to transfer passively the suppressive effect, experiments were carried out to see whether lymph node cells of neonatal rabbits could, under appropriate conditions, show immunologic reaction to rabbit leukocyte antigens by conferring the suppressive effect. Young rabbits (less than 1 week to 3 weeks of age) were injected in the hind feet with 10×10^6 leukocytes pooled from the blood of several adult rabbits. After 4 days the popliteal lymph nodes, regional to the injection site of the leukocytes, were removed from the newborn rabbits and cell suspensions prepared from them were transferred to fresh adult recipients. Two hours after this transfer the same recipients were given antigen-incubated lymph node cells obtained from the original donors of the leukocytes. In addition, some recipients were given only the antigen-incubated cells. As can be seen in Table I, the control recipients developed agglutinins to the usual level of titer, but the recipients given cells of leukocyte-draining lymph nodes in the first transfer showed suppression ranging from marked to complete. The transfer of spleen cells of newborn rabbits injected intravenously 4 days before with 10 or 20×10^6 leukocytes had relatively small effect on the level of agglutinin which developed.

Table II gives a summary of the data obtained on induction of the lymph node cell–suppressive effect by these means in recipient rabbits of the two age groups.

Table II. Summary of data on suppression of transferred lymph node cells in neonatal and older recipients

	Recipient rabbits	
	1 kilogram	*Neonatal*
Ability to support transferred lymph node cells	Yes	Yes, quantitatively not as good as in older recipients
Rejection of transferred lymph node cells in recipients previously injected with donor's leukocytes (active suppression)	Yes	Yes, quantitatively similar to that in older recipients
Capacity of serum produced by injection of leukocytes to transfer passively the suppressive effect on lymph node cell transfer	Yes	Very slight
Capacity of cells of lymph nodes draining sites of injection of leukocytes to confer suppression of transferred lymph node cells	Yes	Yes, quantitatively similar to that of older rabbits

DISCUSSION

In the studies reviewed above, the transfer of lymph node cells has been used as an experimental situation for the study of the immunologic reaction of the rabbit to transplantation antigens within the species. The transfer of lymph node cells incubated in vitro with Shigella antigen had been found to give rise to the appearance of anti-Shigella antibody in the recipient animal, and the immunologic reaction to the transplantation antigens of such cells was indicated by a partial or complete reduction in the amount of the anti-Shigella antibody produced by given numbers of these cells after transfer. In the course of these studies the suppression of antibody formation by transferred lymph node cells was induced in three ways: actively, by the prior injection of prospective recipient rabbits with rabbit leukocytes; passively, by the use of antibody to rabbit transplantation antigens contained in the sera of rabbits which had been injected with rabbit leukocytes; and "adoptively," by the use of rabbit lymph node cells draining sites of injection of rabbit leukocytes. The induction of this homotransplantation reaction (suppression of transferred lymph node cells) by these three methods was examined in young adult and in neonatal rabbits. In the case of the actively induced response, i.e., the suppression of transferred lymph node cells in recipient rabbits which had themselves been injected previously with leukocytes from the prospective donors, there was little difference between the responses of neonatal and older rabbits in relation to absolute amount of leukocyte antigen injected. On the other hand, the concentration of antileukocyte antibody was found to be much lower in sera of neonatal rabbits injected with rabbit leukocytes than in older rabbits injected with the same numbers of rabbit leukocytes.

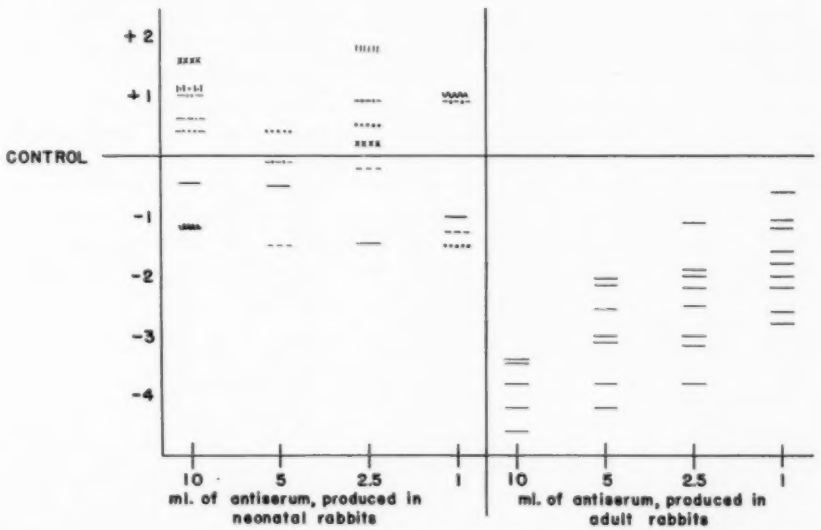

Fig. 5. Effect on transferred lymph node cells of anti–rabbit-leukocyte serum prepared in neonatal rabbits and adult rabbits, respectively. The horizontal line marked *control* presents a reference level, that is, the geometric mean maximal titer of control recipients in each experiment. The horizontal bars indicate the differences, in $\log_2$, between the mean control titer and the mean titer of recipients of lymph node cells incubated with various quantities of the rabbit–anti-rabbit leukocyte serum. The results obtained with serum pools from neonatal rabbits are shown on the left, and those from adult rabbits on the right. The various symbols used for neonatal antisera indicate different serum pools. (From Harris, T. N., and Harris, S.: Ann. New York Acad. Sc. 87: 156, 1960.)

An explanation for the difference in the apparent degree of immunologic response induced in these two ways in these age groups of rabbits might be sought in two directions: First, one could invoke a different immunologic mechanism, one additional to serum antibody, which can operate in the rejection of transferred lymph node cells, and assume that this mechanism is as effective in the neonatal as in the mature rabbit. Second, since a role of serum antibody has been demonstrated in the suppression of transferred lymph node cells, one could postulate that serum antibody is the only immunologic mechanism involved in the suppression of such cells, despite the far lower concentration of such antibody in sera of leukocyte-injected neonatal rabbits. The fact that actively induced suppression is more effective in neonatal rabbits than one would expect from the low levels of antileukocyte antibody could be attributed to a relatively more efficient uptake of circulating antileukocyte antibody during the period before the anti-Shigella antibody appears. An approach to a test of the second hypothesis could be made by an experimental situation in which a shorter interval is allowed for the production of the suppressive antibody or for the cumulative action of such antibody on transferred cells. Further study will be required to see whether the data on the suppressive reaction in the actively immunized neonatal rabbit can be explained in terms of antileukocyte antibody as the sole mechanism of immunologic reaction by the rabbit to antigens of transferred lymph node cells.

SUMMARY

The transfer of rabbit lymph node cells incubated in vitro with antigenic material derived from *Shigella paradysenteriae* to recipient rabbits leads to the appearance of agglutinins to Shigella in the sera of the recipient animals. The production of the antibody by the transferred cells is suppressed if the recipient rabbits have received a prior injection of leukocytes obtained from the donor animals.

The suppression of antibody formation by transferred lymph node cells has also been demonstrated when the transferred cells have been incubated with serum obtained from rabbits which have been injected with rabbit leukocytes. This is presumably due to the effect of antibodies to leukocyte antigens within the heterogeneous population of rabbits.

The transfer of lymph node cells to neonatal rabbits is also followed by the appearance of antibody in the sera of these animals. In the case of the neonatal recipients the suppression of transferred lymph node cells can also be actively induced by the prior injection of the donors' leukocytes; in the induction of this effect given numbers of rabbit leukocytes are almost as effective in the neonatal as in the adult recipient rabbit.

The suppressive effect can also be conferred on recipient rabbits by injecting them with other rabbit lymph node cells—in this case cells of nodes draining sites of injection of rabbit leukocytes. Here, again, cells of neonatal rabbits injected with rabbit leukocytes are as effective as those of adult rabbits in the suppression of antibody formation by transferred lymph node cells.

The sera of neonatal rabbits injected with rabbit leukocytes are, however, far less effective than are similar sera from adult rabbits in passively transferring the suppressive effect on transferred lymph node cells. Two possible bases for this difference are indicated.

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Virus-induced acquisition of metabolic function

A model for studies of inheritance and differentiation

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IN THEIR beginning, our chemical studies of the infection of *Escherichia coli* by T_2, T_4, and T_6 bacteriophages were directed to an understanding of the parasitic mechanisms and extreme virulence of these viruses.[1, 2] These studies also permitted us to devise some approaches to the chemotherapy of virus infection.[3] The major clues to our present insight to parasitism and virulence with the T-even phage systems stemmed from our discovery of the unique pyrimidine, 5-hydroxymethyl cytosine (HMC) in these viruses.[4] It is now clear that the formation of this unique structure in the infected cell converts one metabolite, deoxycytidylate, essential for the synthesis of normal host DNA, to another, 5-hydroxymethyldeoxycytidylate (dHMP), essential for the production of viral DNA. By this and a few other simple chemical devices, a normal cell is diverted entirely to virus production. The viral DNA, because of the presence of the hydroxymethyl group in HMC can accept glucose residues which help to protect viral DNA against the nucleases which do destroy the host DNA.[2] Finally, T2, T4, and T6 differ among themselves precisely in the numbers of glucose residues in their DNA, thereby accounting in some measure for speciation within this group of viruses.[2]

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**The series of studies described in this paper was carried out in largest part with the aid of grants from the Commonwealth Fund. The studies of bacteriophage systems begun at The Children's Hospital were the first systematic inquiries into the chemistry of host cell-viral interactions; they have provided many useful lessons for studies into other types of viral infection. However, the first decision that such studies with bacteria and bacteriophages were an appropriate subject of exploration in a department of pediatrics and in a children's hospital was made without any detectable hesitation by Dr. Joseph Stokes. His continuing understanding and encouragement of our aims, hypotheses, and work were an important stimulant to our progress.*

VIRUS INFECTION AND THE GENETIC CONTROL OF PROTEIN SYNTHESIS

Although this viral infection is unique in these chemical details, analysis of the mode of origin of the unique viral pyrimidine, HMC, rapidly embroiled us in the major problem of the genetic determination of protein synthesis. The synthesis of the normal ubiquitous DNA pyrimidine, 5-methyl uracil or thymine, had been clarified by Friedkin and Kornberg,[5] as in *a* of Fig. 1. In this system a tetrahydrofolate complex of formaldehyde transfers the latter unit to uracil and simultaneously effects a re-

duction to form thymine. Flaks and I[6, 7] then proceeded to find a new enzyme in infected cells, one which catalyzed the reaction in *b* of Fig. 1, i.e., the addition of formaldehyde to deoxycytidylate in the presence of tetrahydrofolate to produce the viral pyrimidine in the deoxyribonucleotide, dHMP. We also showed that infected cells contain a great deal more thymidylate synthetase than in uninfected cells and demonstrated a new important inhibitor for this enzyme, 5-fluorodeoxyuridylate.[8]

The enzyme which forms hydroxymethyl deoxycytidylate is extraordinary in that it is present only in virus-infected bacteria.[9, 10] The enzyme has been purified considerably,[10] although not completely as yet, and appears to have a molecular weight of about 65,000. Using kinetic data obtained on the purified enzyme, we have shown that there is not even a single active enzyme molecule in an uninfected bacterium.[10] This is the first demonstration of the complete absence of an enzymatic activity in genetically deficient cells.

The appearance of the enzyme requires insertion into the host cell of the viral DNA containing HMC. With the use of ultraviolet irradiated virus, it has been shown that the size of the portion of the DNA controlling production of this enzyme is very much smaller (perhaps a twentieth) than the total DNA of the virus.[9, 11]

The total DNA of the T-even phage also carries information for the development of other enzymes, e.g., the thymine-synthesizing enzyme,[12] whereas a non-HMC containing virus such as T5 can induce the development of the latter enzyme but not of the hydroxymethylase.[13] Some other bacterial viruses such as T1 will not stimulate the production of either enzyme.[13] Thus, the picture has emerged of viral DNA as a linear array of many codes, each controlling production of a particular enzyme. T2 with more DNA and, therefore, possessing more codes controls the synthesis of more enzymes than does T5 which in turn transmits more information than does T1.

It is our belief that the new enzymatic activities which appear are associated with new protein molecules whose biosynthesis and specificity are determined by the insertion into the host of the new viral DNA molecules. The old codes are destroyed at the inception of infection by degradation of host DNA. That the appearance of the new enzymatic activities does indeed represent biosynthesis rests on the following evidence:

1. These enzymes cannot be found in virus and constitute a greater mass than is present in a single T2 particle.

2. The enzymes do not appear to be masked in uninfected cells, i.e., a masking agent or inhibitor of the enzymes cannot be detected in extracts of normal cells.

3. The appearance of the dCMP hydroxymethylase and the thymidylate synthetase requires protein synthesis[9, 10, 12] and is inhibited by conditions of amino acid de-

a. The biosynthesis of thymidylate (dTMP) from deoxyuridylate (dUMP)

$$\text{dUMP} + \text{HCHO} + \text{tetrahydrofolate} \xrightarrow[\text{from normal or infected cells}]{\text{enzyme}} \text{dTMP} + \text{dihydrofolate}$$

b. The biosynthesis of hydroxymethyl deoxycytidylate (dHMP) from deoxycytidylate (dCMP)

$$\text{dCMP} + \text{HCHO} + \text{tetrahydrofolate} \xrightarrow[\text{from infected cells only}]{\text{enzyme}} \text{dHMP} + \text{tetrahydrofolate}$$

Fig. 1. Reactions in the biosynthesis of pyrimidine deoxyribonucleotides of T-even bacteriophage.

ficiency or by amino acid analogues. The production of enzyme occurs within 1 to 2 minutes after infection and continues at a constant rate for 10 to 15 minutes, after which time enzyme production ceases. Preparations of purified enzyme contain exogenous isotopic precursor added after infection. The purified enzyme has not been separated from such protein-bound labeled precursors.[10] Thus, no evidence has been obtained for preformed bacterial precursors of the enzymes.

4. The appearance of enzyme requires prior RNA synthesis.[14] This can be blocked by establishing a uracil deficiency in certain infected bacterial strains.

In summary, the present evidence strongly suggests that portions of viral DNA induce the production of specific RNA molecules which direct the production of the various enzymes which are made after infection. It is evident that this system is behaving in a manner completely consistent with the current hypotheses of the genetic control of protein synthesis and its intermediation, presented in Fig. 2. However, these systems are not only consistent with other systems in these respects but they also are among the most favorable systems for further clarification of these phenomena. This is true because in infection with the T-even viruses biologically homogeneous units of DNA containing a mass of about a fiftieth that of the bacterial DNA may be inserted almost simultaneously into the bacterial host. The rapid destruction of the bacterial genome prevents the continuing elaboration of normal bacterial proteins and nucleic acids, and the entire metabolic machinery of the cell is thrown synchronously into the production of the limited number of proteins now known to be produced in virus infection. These properties of simultaneity of entrance of new genetic units, cessation of synthesis of normal protein, synchrony of the host's metabolic equipment, and magnification of the synthesis of the qualitatively new proteins are clearly favorable attributes for the study of the physiology and mechanism of genetically determined protein synthesis.

After our initial demonstration of the biosynthesis of some new enzymes in virus infection, our observations have been extended by a number of laboratories, of which only a few can be mentioned.[15-18] It is now possible to describe the production of a large number of proteins, both enzymes and virus proteins, as in the summary in Fig. 3. The reactions, *1* through *13*, describe steps in the biosynthesis of viral DNA, represented by either qualitatively new enzymes or the stimulated production of old activities in markedly increased amount, e.g., the dGMP kinase, etc. In the latter category of proteins synthesized it appears that even the old activities are now effected by new types of proteins amounts of newly recognized proteins, i.e., the viral DNA produces qualitatively different proteins bearing active catalytic sites similar to those in the previously synthesized proteins. These enzymes *(1* to *13)*, possibly the DNase *(16)*, and the internal phage "gut" protein *(14)* all of which are enumerated in Fig. 3, are made in the first minutes after infection, and if they are not produced, viral DNA is not made. The frenetic activity which takes place in these first few minutes certainly focuses on fewer proteins than does biosynthesis in the uninfected cell. Nevertheless, it is clear that we still have to unscramble the synthesis of a dozen or more proteins in the early stages of infection and it should be asked whether even simpler systems for the study of protein synthesis are not available.

VIRUS INFECTION AND CHOICE IN THE BIOSYNTHESIS OF PROTEINS

The proteins (*1* to *14*) described at the top of Fig. 3 are elaborated early in infection. With the exception of the phage internal protein *(14)*, their continuing production is unnecessary for phage multiplication since the amounts of these enzymes are adequate for the observed rate of DNA synthesis at the time this synthesis starts, i.e., at about 7 minutes after infection. This sufficiency is efficiently noted by the infected cells: without breaking stride in the rate of protein synthesis they switch protein synthesis,

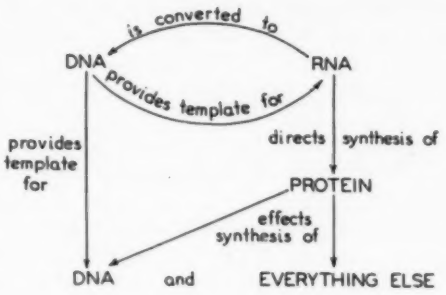

Fig. 2. General biosynthetic interrelations of the nucleic acids and proteins.

shortly after DNA synthesis begins, to the elaboration of a new class of proteins, the viral structural proteins (*17* to *23*). How does a cell containing its full complement of genetic data alter one path of protein synthesis and embark on another? This question may be thought of as one important aspect of the problem of differentiation, and its clarification in this system should help to answer this and similar questions for more classical differentiating systems. We are not very far advanced on this problem but have begun to obtain some relevant data and, more importantly, have perhaps begun to pose the proper questions.

Although the synthesis of DNA is markedly stimulated in infected cells, the total amount of RNA does not increase significantly.[1] Nevertheless, isotopic techniques have demonstrated that a small amount of RNA is made immediately after infection.[19] This RNA has a base composition which mimics that of the virus DNA[19] and as we have shown is required in the production of the phage-induced enzymes.[14] This RNA has a high rate of turnover early in infection, i.e., the atoms incorporated within it during biosynthesis subsequently disappear from this fraction.[19] Indeed, the nucleotides of the virus-induced RNA subsequently appear in virus DNA. We have postulated, therefore, that the switching off of enzyme synthesis is the consequence of the degradation of the critical RNA templates. It appears further that the nucleotides derived from this RNA are also used most efficiently as important precursors of the deoxyribonucleotides.

A number of important questions can be asked of this phenomenon. Are all of the RNA molecules produced in phage infection degraded, and if so, by what means? Can the conversion to DNA occur in the intact RNA molecules or even at their ends? How are deoxyribonucleotides synthesized? In attempting to answer these questions we have

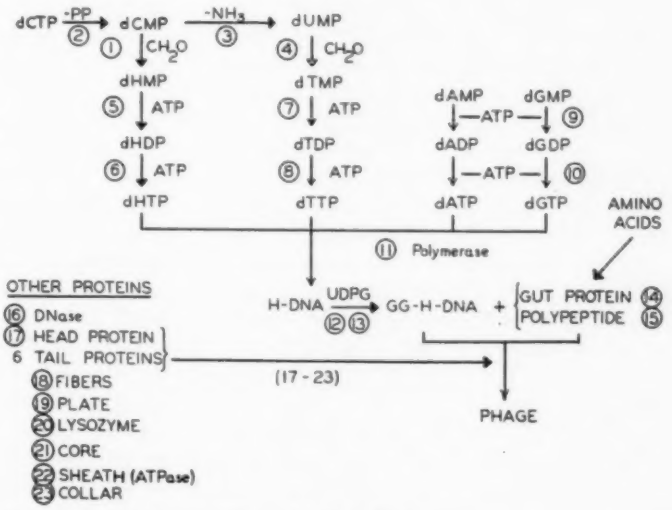

Fig. 3. New and stimulated reactions in T_6 infection.

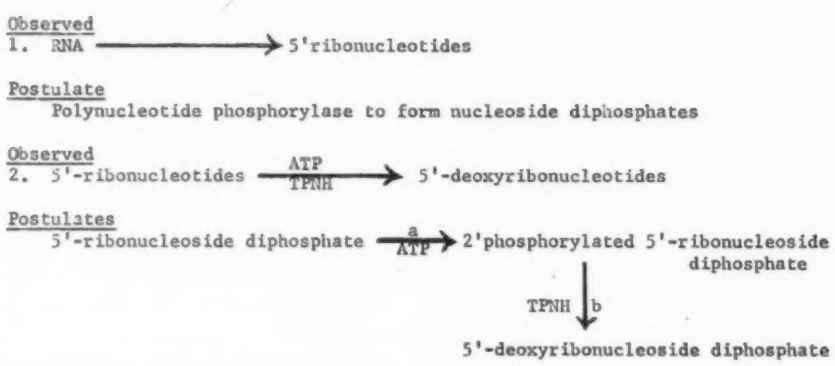

Fig. 4. Conversion of phage-induced RNA to deoxyribonucleotides.

explored the behavior of extracts of phage-infected cells.[20] We have prepared extracts of infected cells which were permitted to incorporate an isotopic RNA pyrimidine, uracil-C,[14] after infection. The uracil entered a chemically distinct fraction of RNA, shown to be only 4 per cent of the total RNA of the cell. It also entered into the acid-soluble nucleotide fraction and into a small amount of viral DNA. When an extract was prepared and allowed to incubate, about 50 per cent of the isotopic (virus-induced) RNA disappeared and the isotope appeared in the acid-soluble fraction. During this process, very little of the unlabeled bacterial RNA was lost. Thus, about half of the viral-induced RNA was selectively hydrolyzed without significant degradation of host RNA. It could be shown by end group estimations that the degraded molecules were destroyed essentially in toto. It appears likely that the degrading enzyme is the formerly mysterious polynucleotide phosphorylase which seems to attack the normal bacterial RNA much less readily than the phage-specific RNA, the latter being hydrolyzed to ribonucleoside-5′-diphosphates. Since about half of the phage-induced RNA is left intact, we may wonder if this residual RNA fraction does not contain the codes for the synthesis of the virus structural proteins.

If the extract is fortified with ATP and the reduced coenzyme, TPNH, the system produces deoxyribonucleotides in about a 75 per cent yield calculated from the RNA which disappears.[20] However, deoxyribonucleotide production may occur, starting with phage-inducing RNA or with the acid soluble nucleotide fraction. In the former instance, addition of a nonisotopic ribonucleotide pool to the labeled RNA reduced the rate of appearance of labeled deoxyribonucleotide, indicating a hydrolysis of the RNA prior to conversion of the low molecular ribonucleotides to deoxyribonucleotides. These results and our conclusions are summarized in Fig. 4.

This formation of deoxyribonucleotides from ribonucleotides occurs in infected cells at a rate about 20 times greater than that reported earlier for extracts of uninfected cells.[21] This observation suggests that the enzyme or enzymes for this conversion have also been synthesized after infection. We are now exploring the details of this process in order to test whether new catalysts of this type have actually been formed. We are studying the conversion of the defined isotopic substrate, cytidine-5′-phosphate to deoxycytidine-5′-phosphate (dCMP) and have also observed an unusually high rate of reaction in extracts of infected cells for this more limited system.

From these studies of phage multiplication it appears possible that a cell containing a full genetic complement can develop qualitatively distinct phases of protein synthesis by the expedients of producing several classes of RNA molecules and of using these different classes of RNA at different times. A

number of mechanisms may be employed to determine the origin and fate of these molecules. We may suppose that the entire complement of intermediary RNA is made initially and that only that half which is sensitive to degradation can take precedence in the initial phase of protein synthesis. As an alternative we may suppose that only the RNA used in the initial phase is made for enzyme synthesis and that a new class of RNA molecules is made subsequently for synthesis of virus proteins. Either mechanism poses an important set of questions.

However, in addition to these questions, a large number of others may be posed, which as a group are of general significance for our understanding of differentiation and development.

1. How is the choice made to use the genetic complement for DNA synthesis or for RNA synthesis? This basic choice presumably determines the apparent antithesis between dividing and differentiated cells.

2. When RNA is made, how is it determined that this shall be permitted to direct protein synthesis rather than be degraded to ribonucleotides?

3. How is it determined that ribonucleotides will be used for RNA synthesis rather than be converted to deoxyribonucleotides, or vice versa?

4. If TPNH is essential to the latter reaction and is also essential to numerous biosyntheses, such as fatty acid synthesis, hydroxylation of steroids, iodination, etc., how does a cell dole out this critical metabolite? Could not this simple competitive relation determine whether a multiplying tissue culture cell stop multiplying and begin to accumulate fat droplets as a first sign of the deterioration of the culture? Or could the overavailability of TPNH in muscular dystrophy be a major cause of the overabundance of DNA synthesizing and multiplying elements in previously nonmultiplying tissues?

These questions which are evidently of general import, both theoretically and practically, can in some measure be answered in the system of *E. coli*-T bacteriophage. At the present time the infected bacterium may even be the system of choice for certain facets of this exploration, as it has been for many other biologic problems in the past.

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The changing aspects of the serodiagnosis of viral infections

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WHEN the first virus diagnostic laboratories were established some 15 years ago, relatively few viruses and rickettsiae had been recognized as causes of human illnesses, and not all of these could be handled readily in the laboratory. Under these limited conditions it was thought that a specific laboratory diagnosis could be rendered most conveniently by serologic tests; i.e., by the demonstration of a rise in specific antibodies to one of the viral antigens between the acute and convalescent stages of illness. The incidence of positive answers was, of course, low because of the limited number of antigens available at that time. Moreover, as it turns out now, the answers probably were not always correct, in view of cross-reactions and recall phenomena to be discussed below. Isolation of the etiological agent from specimens of patients was de-emphasized in earlier times because of the difficulties and frequent failure of the available procedures.

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This general situation has changed drastically in the intervening years and isolation of the virus should now constitute the primary approach to virus diagnosis. The reasons for this change in approach are several: (a) The introduction of the suckling mouse as a host and of tissue culture techniques into virology has increased considerably the potential of successful isolation of the virus and with it the direct demonstration of the presence of a pathogen in the patient, which obviously affords the most significant information. (b) The introduction of these new techniques has facilitated not only the handling of some of the "older" viruses, but has led also to recognition of numerous new viruses or groups of viruses as causes of human illness. The Coxsackie A and B, the ECHO, and the parainfluenza groups of viruses each consisting of multiple serologic types, are examples to the point. Many of these agents can cause disease entities which are indistinguishable on clinical grounds, and, as a result, dozens of viral antigens would have to be employed now for serodiagnostic tests on individual patients. The multiplicity of etiological possibilities and the corresponding requirement

of numerous specific antigens have rendered routine serodiagnosis to some extent impractical. (c) More important, quite a few antigenic crossings have become apparent within and sometimes also between groups of viruses. Some of these interrelationships may be directly detectable in the cross-matching of antigens with specific homologous and heterologous antisera prepared by immunization of experimental animals. Others may become apparent only by recall of antibodies or by a general broadening of antibody responses after previous exposures to other members of a given group of viruses. Such recalls have often rendered interpretation of diagnostic tests difficult, if not impossible. The first two points require no discussion, but the third needs amplification.

Viruses are, as a rule, composed of more than one antigen and some reveal a complex antigenic structure. Cross-reactions may be based on the presence of distinct antigens in some instances or on closely related antigenic configurations in others. Generally, one would expect specific properties of a virus, which serve to differentiate it from other agents, to be reflected also in a specific antigenic component. While this is true in most cases, in others the specific antigen can be demonstrated only with difficulty or not at all. A separation of specific and common antigens would seem to be desirable and is indeed essential in some instances for demonstration of significant diagnostic antibody responses. The common antigens would seem to provide for simplification of serodiagnostic procedures in that they would permit a "group" diagnosis. Antibody responses to the specific antigens would then serve to identify the infecting agent. In reality, clean-cut separations of common and specific antigens have rarely been achieved, because in many instances cross-reactions may not be based on physically separable components but rather on related antigenic configurations.

In order to illustrate some of the points made above, the myxovirus and enterovirus groups have been singled out for detailed discussion. The myxovirus group has perhaps been most extensively studied in this respect, and, therefore, will be considered first.

SEROLOGIC RESPONSES IN MYXOVIRUS INFECTIONS

Influenza virus. It has long been known that tissues infected with the virus of influenza contain two complement-fixing antigens. One is identical with the virus particle and called virus, or V antigen, and the other is smaller, separable from the virus particle, and referred to as soluble, or S antigen. It was shown that S is antigenically distinct from V and that it is common for all strains of one type, but the S antigen of Type A is different from that of Type B. For a long time, it was thought that the S antigen represented merely a product of host–virus interaction since it was found in abundance in infected tissues but not in purified virus suspensions. However, Hoyle[1] demonstrated that S also forms an integral part of the virus particle in that, on exposure to ether, the virus particles disintegrate with release of internal S antigen. The S antigen is a nucleoprotein[1, 2] of the ribonucleic acid (RNA) variety, the only nucleic acid-containing component of the virus, and thus must contain its genetic information. Influenza virus particles of all strains contain comparable amounts of S antigen as long as they are fully infectious.[3] Under certain conditions of cultivation, noninfectious virus particles are obtained. Such incomplete virus particles contain less RNA than fully infectious particles[4] and less, or no, detectable S antigen.[5] Electron micrographs of thin sections of such particles reveal a less dense internal stucture than infectious particles, or none at all.[6] These data indicate that the S antigen is probably the most important part of the virus, that it is internally located, and that therefore antibodies to the S antigen fail to prevent infection.

The V antigen forms the outer shell of the virus; it is a protein or possibly a lipoprotein. It cannot be separated from the hemagglutinating activity of the virus and indeed shows the same strain-specific orientation as the latter.[7] After treatment of

the virus with ether, hemagglutinating particles of about 35 to 50 mμ diameter are obtained[8, 9] which reveal some changes in the degree of interaction with red cells of chickens and guinea pigs as compared to intact virus particles,[3] but the V antigen remains unaltered with respect to its specificity.[7]

It is evident then that the S antigen is specific for the type of the virus and that the V antigen serves to differentiate between subtypes and strains. Both reagents have their place in the serodiagnosis of influenza, and standard procedures for the production of specific antigens and control antisera have been worked out.[10] The S antigens of Types A or B are useful in the serodiagnosis as to the type of the infecting agent, which in most instances would be sufficient—especially during epidemics—once the offending strain has been identified. The fact remains, however, that children under the age of 3 years, sustaining presumably their first infection with an influenza virus, fail to form anti-S in about 50 per cent of the cases.[11] In these instances only the V antigen of the homologous strain or a close relative of it can render the serologic diagnosis. Anti-V of course develops in all cases but the responses are complex.[12] The patients not only form antibodies to the V antigen of the currently infecting virus; they also form recall antibodies to the V antigens of strains of the same type with which they have had previous experience. The recalled antibodies appear earlier and may reach higher titers than the antibodies to the V antigen of the infecting virus. Appearance of the latter may, on occasion, be delayed as long as 3 weeks after the onset of illness.[11] It must be emphasized that such recalls of anti-V are induced even by viruses which show no detectable antigenic cross-relationships when tested with specific animal-immune sera. Furthermore, many patients form antibodies to V antigens of strains to which they could not have been exposed because they were either circulating before the individuals were born or the sera were collected before the strains became prevalent.[12] Thus, some children infected with A_2 virus during the 1957-1958 pandemic produced antibodies to early A strains of the 1930's; or patients in the last A_1 epidemic in 1957 formed antibodies to A_2 which, at that time, had not emerged.

These various results suggest that a strain-specific antibody response, which reflects solely the infecting virus, may be obtained only on first exposure to a virus of influenza. With subsequent exposures to other homotypic strains not only are antibodies to the earlier infecting viruses recalled, but a general broadening of the antibody spectrum may be observed which embraces strains to which the patient could not have been exposed. Such broadening may be observed on occasion even after a single severe infection in infants with pulmonary involvement in which the concentration of virus may reach unusually high levels. The basis for these responses is not as yet clear. One could assume that each strain of virus of one type contains many if not all, of the various antigenic configurations. Some of these are dominant in any given year and determine the strain specificity; others are minor in concentration, responsible for directly detectable cross-reactions, usually within a given subtype; and the remainder are present in trace quantities, sufficient to recall antibodies or to induce antibody formation after repeated or heavy exposures to them. It is also conceivable that, in time, less specific antibodies are formed, which fit partially to many antigens.

While the example of influenza provides a relatively well-documented example of the recall phenomenon and of broadening of antibody responses in successive infections over the years by members of a group of viruses, no especially disturbing complications are encountered in this particular case in the serodiagnosis, because, with the appearance of a new strain or subtype of influenza, the preceding one rapidly disappears and only one remains in circulation for a given time. Thus, a rise in antibodies to S antigen, or to any of the V antigens of a given type to all intents and purposes denotes an infection with the current strain.

Nonetheless, since all patients do not uniformly recall antibodies to all strains, it is desirable to employ for diagnosis the current strain or a close relative of it as antigen.

Mumps and parainfluenza viruses. The specificity of the serologic diagnosis of mumps by complement fixation test has remained unchallenged for a long time, yet recent work has brought forth certain relationships between mumps and parainfluenza viruses which require some consideration.[13]

As in influenza, a soluble, S, and a virus, V, antigen can readily be differentiated.[14] In this instance, antibodies to the S antigen, as a rule, rise earlier than antibodies to the V antigen and the latter persist for a longer period than the former.[15] The differential appearance of antibodies permits an early presumptive diagnosis in about 70 per cent of the cases on the acute stage serum in that an elevated anti-S titer and little or no anti-V are found only in the first days of illness. A subsequent rise in anti-V serves to confirm the presumptive diagnosis.

The diagnostic reliability appears to remain of a satisfactory order, especially in conjunction with clinical and epidemiologic probability. Problems are encountered, however, in the case of the complement fixation technique for determination of susceptibility. Such tests require mainly the V antigen, since, after infection, the anti-S antigen declines to subdetectable levels more rapidly than the anti-V. While such tests have shown a high degree of reliability,[16, 17] failures have been noted in that a small percentage of individuals, who were judged immune on the basis of positive complement fixation tests, developed mumps on subsequent exposure. It is conceivable now that some of these "false immune reactions" were based upon cross-reacting antibodies evoked by infections of the parainfluenza virus.

As was shown recently by Chanock and his associates,[18] infection by parainfluenza viruses may frequently lead to a rise in heterotypic antibodies. This was especially evident in patients from whom Type 1 virus had been isolated. Many of these developed antibodies to Type 3 virus, sometimes even unaccompanied by a homotypic response. Such results, which most likely reflect recall phenomena, obviously prevent a reliable serodiagnosis of infections by this group of agents.

SEROLOGIC RESPONSES IN ENTEROVIRUS INFECTION

The enterovirus group comprises the poliomyelitis, Coxsackie A and B, and the ECHO viruses which are subdivided into 3, 24, 6, and some 30 types, respectively. Relatively little work has been done with respect to the antigenic structure of the Coxsackie and ECHO viruses except to show that they can be differentiated as to type in complement fixation tests with specific animal-immune sera, and that cross-reactions, if noted, generally are of a minor nature. The antigenic structure of the virus of poliomyelitis has been studied more extensively.

Preparations of the virus of poliomyelitis of one type obtained by infection of cell cultures contain, as a rule, two type-specific components aside from some group-specific activities which, as yet, have not been clearly defined. The relative concentrations of the type-specific antigens depend to some extent upon the cells and media used for cultivation of the virus, the length of the incubation period, and the handling of the antigens after harvest. It was shown by Mayer and his colleagues[19] that highly purified and concentrated preparations of virus could be separated by centrifugation procedures into two serologically active fractions. The heavier fraction, which was found to contain most of the infectious virus, has been referred to as the D component, and the other, which contained little infectious virus, as the C component. Certain human sera were found which reacted either only with D or only with C, but most sera interacted with both. It was shown that two distinct type-specific antigens are demonstrable in infected cell cultures without extensive concentration procedures.[20] These studies were greatly simplified by prior treatment of the virus

preparations with fluorocarbon,[21] which removes anticomplementary and host components without significantly affecting some viral antigens. When the virus preparations were harvested early, i.e., at the time when cellular destruction had just reached completion, one principal antigen was found which appeared to be identical with the D fraction.[20] On heating at 56° C. for 30 minutes this antigen was completely converted into another antigen, which now behaved serologically like the C fraction.[20, 22] Antigen of this type also appeared in increasing concentrations in virus preparations which were harvested after prolonged incubation periods. The native type of antigen has been called N, and the heated one, H antigen. A different terminology appeared to be justified because the H antigen, converted from N by heat, is physically different from the C component in that it cannot be separated from N by centrifugation procedures. The distinct serologic specificities of N and H could be demonstrated in several ways. Absorption of certain type-specific human sera with H left antibodies to the N antigen behind, and, conversely, absorption with N failed to remove the anti-H antibodies.[20] Furthermore, occasional human sera reacted only with N or only with H. Such specimens were found rarely, however, and it became essential to produce, if possible, specific anti-N and anti-H sera in experimental animals. This was achieved in guinea pigs.[23] On repeated intraperitoneal injections of heated virus these animals formed only type-specific anti-H. Immunization with native virus induced antibodies to type-specific N as well as H, and the latter antibody was then removed by absorption with homotypic H antigen to obtain a specific anti-N serum.

The availability of specific anti-N and anti-H guinea pig sera now permits accurate standardization of N and H antigens and determination of their specificities. N antigens are readily freed of "contaminating" H by passage through cellulose columns (DEAE),[24] and H antigens are obtained merely by heating of viral preparations at 56° C. After column chromatography of N antigen of Type 1, the various fractions obtained were examined for the number of plaque-forming units (p.f.u.) present as determined by assay on monolayers of susceptible cells, complement-fixing activity, and physical virus particle content (P.V.P.) as counted under the electron microscope.[24] The P.V.P./p.f.u. ratios varied from about 50 to 4 in the various fractions. In the preparations with the lowest P.V.P./p.f.u. ratios, one N antigen unit corresponded to about 10^7 virus particles. This P.V.P./N antigen unit ratio is about 1,000 times lower than the P.V.P./D antigen ratio determined by Mayer and co-workers.[19] Similar studies on the H component have thus far failed because it has not been possible to obtain complete recovery of this antigen from DEAE columns.

The results obtained by column chromatography indicate that the virus populations obtained from cell cultures are extremely heterogeneous with regard to their infectivity and surface properties. It has long been known that denaturation of proteins may result in qualitative changes in antigenicity, although most procedures employed were rather drastic or effective only at the isoelectric point. It is conceivable that milder methods, such as heating at 56° C. at neutral pH, may affect such a change and convert N to H rather than destroy the heat-labile N-antigen and, thereby, may expose the heat-stable H component. It must be pointed out, however, that both antigens appear simultaneously in infected cells.[25] It would seem to be unlikely that synthesis of poliomyelitis protein is followed immediately by some measure of denaturation. One may assume rather that H is a precursor of N and that heating may reverse the final step in its production, possibly by the breaking of certain chemical bonds.

Thus far, only type-specific reactions have been considered. The guinea pig sera, produced as described, failed to show cross-reactions with other types. Yet studies with many human sera indicated that heterospecific antibodies do exist and therefore pre-

sumably also antigens common to two or all three types of poliomyelitis virus (see below). Since it is unlikely that such antibodies result from a single infection, preliminary experiments were carried out in which guinea pigs were injected simultaneously with Types I and II viruses.[26] Their sera revealed, after appropriate intervals, not only antibodies to the N and H antigens of the two injected types, but also to the H antigen of Type III. The anti–Type III H antigen could be removed from the serum by absorption with Type I or II H antigen.

The use of the type-specific N and H antigens has, for the most part, not been successful in improving the serodiagnosis of poliomyelitis in man because of the frequency of heterotypic responses, which may not even be restricted to poliomyelitis antigens but may also include those of other enteroviruses. Monospecific responses are rare and found mainly among very young children. It appears that, as in influenza, only the primary infection with a member of the group may lead to a strictly type-specific antibody response, a rise in antibodies to the H antigen preceding that of anti-N by several weeks. Indeed, first serum specimens, taken early after onset of paralysis, often contain already significant titers of anti-H to the infecting type of virus which may increase only a little or not at all in subsequent weeks. Only the N antigen may reveal a diagnostically significant rise in antibodies under such conditions. The antibody responses, after immunization with live attenuated poliomyelitis vaccine, generally follow a similar course,[26] but upon injection of formalin-inactivated vaccine anti-H and anti-N develop nearly simultaneously.

With advancing age the likelihood of previous exposures to other poliomyelitis and enteroviruses increases and with it, apparently, the complexity of antibody responses to new infections. Rises in antibodies are noted not only to the currently infecting poliomyelitis virus but also to the N and/or H antigens of one or both of the heterotypic agents. These heterotypic responses may reflect recalls or a general broadening of antibody spectra or antibody reactivity on repeated infections with one or more of the viruses. The latter suggestion is based on the observation that absorption of broadly reactive human convalescent sera with N or H antigens of one type may, to a variable extent, remove heterotypic antibodies.[20] For example, after a febrile illness associated with muscular pain, the serum of one adult patient showed a marked increase in neutralizing and complement-fixing antibodies to the N and H antigens of all three types. All anti-H was absorbed by any of the H antigens, but the neutralizing and anti-N activities remained unaffected. Absorption of the same serum with N antigens of Types I and III removed only the homologous neutralizing and complement-fixing antibodies, but N antigen of the Type II, reduced, in addition, the heterotypic neutralizing antibodies and anti-N titers. Such recall phenomena and broadening of antibody responses do not seem to be restricted to infections with poliomyelitis viruses but seem to include other enteroviruses as well. For instance, patients from whom a Coxsackie B virus was recovered may show not only an antibody response to the isolated agent but also to other Coxsackie B and even to some of the poliomyelitis and ECHO virus antigens.

These subtle interrelationships among the enteroviruses deserve further study. As in the myxovirus group, it is not clear whether the various agents actually share antigenic components or whether they possess closely related antigenic configurations sufficient to induce recall phenomena or whether, after multiple experiences with various members of the group, antibodies of broader specificity are formed. Whatever the answer may be, these responses have rendered the serodiagnosis of enterovirus infections largely unsatisfactory.

DISCUSSION

The examples cited serve to illustrate the problems encountered nowadays in the serodiagnosis of viral infections. These problems are by no means restricted to the comple-

ment fixation technique or to the groups of viruses singled out for discussion. It suffices to say here that recall phenomena and broadening of antibody spectra may be seen also in hemagglutination inhibition and neutralization tests and that the strain-specific or type-specific serodiagnosis of infections by members of the psittacosis-lymphogranuloma, pox, adeno-group and arthropod-borne A and B groups of viruses is beset with similar difficulties.

While it is often not possible to demonstrate cross-reactions between members of given groups of viruses in direct in vitro analyses with specific animal-immune sera, multiple experiences with members of a group change the responses of animal and man considerably and cross-relations among viruses may become apparent only in this fashion.

In view of these experiences considerable caution must be exercised in the interpretation of serodiagnostic tests; indeed, interpretation often is no longer possible. This dilemma has made it clear that attempts at isolation of virus must now form the primary approach to the diagnosis of the virus. Serologic tests remain, however, an important function of virus diagnostic services. They are essential, or at least useful, for: (a) confirmation of results of virus isolation since the patient should develop antibodies to the isolated agent; (b) diseases caused by essentially "monospecific" agents, such as the rickettsiae of Q fever and the viruses of lymphocytic choriomeningitis and herpes simplex; (c) infections by a virus known to be the only member of a group active in a given geographic area, such as Eastern equine encephalomyelitis virus along the eastern seaboard; (d) groups composed of members which each cause distinct clinical entities, such as the psittacosis-lymphogranuloma group; (e) early childhood infections in which the illness under study may constitute the first experience with any member of a given group; and (f) circumscribed outbreaks of disease once the offending agent has been identified. One may add that where attempts at isolation of the virus fail, serology may still provide an answer or at least a hint as to the group of viruses involved.

While the above remarks all point to the need for increasing efforts toward recovery of the etiological agent, even success of isolation is not without problems of interpretation. There is little question that pathogens obtained from the sites of infection bear a causal relation to the disease under study, such as the isolation of herpes simplex virus from brain tissue, or a Coxsackie B virus from the spinal fluid, or psittacosis virus from the sputum. The relationships are not as definite when an agent is isolated from the stools or throat swabs in infections of the central nervous system. When the virus of poliomyelitis is recovered under such circumstances, few tend to doubt its significance. With isolation of a Coxsackie or ECHO virus, interpretations are more guarded and the possibility of a "passenger" virus, not related to the disease, must be considered, since inapparent infections with these agents are frequent. Indeed, in situations where the severity or type of clinical disease, according to present knowledge, does not seem to be compatible with the virus isolated, the suspicion should be entertained that an additional, more virulent virus was in fact responsible for the disease. On inoculation of mixtures of viruses into host systems, the agent which is present in excess or which multiplies at a faster rate may outgrow the other, sometimes to its apparent exclusion. Such interference phenomena are not uncommon. The excluded virus can be demonstrated only by neutralization of the interfering agent by specific-immune sera prior to inoculation. To illustrate this point, a case of severe paralysis may be cited: The stool of a patient yielded Coxsackie B_3 virus, which hitherto had not been implicated in paralytic disease. When the stool suspension was retested after addition of specific anti-Coxsackie B_3 serum, a poliomyelitis Type I virus was isolated. Interference, however, is not the universal outcome of dual infections and, on occasion, two viruses may be isolated directly from the same patient. In these

cases, it might be difficult to decide on the role played by either one in the disease under study. Furthermore, one should not overlook the possibility that two viruses may act in concert to produce a more severe illness than generally seen with either one alone.

SUMMARY

Problems in the serodiagnosis of viral infections have been discussed and illustrated by appropriate examples. While the first infection with a member of a given group of viruses often leads to a strain-specific or type-specific antibody response, subsequent infections with other members of that group may recall antibodies from earlier experiences and lead to a general broadening of antibody spectra. In the face of such multiple antibody responses, a specific diagnosis cannot be made unless the offending virus is isolated from the patient. Attempts at isolation of virus should, therefore, constitute the primary approach to the diagnosis of viral infections.

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The response of blood glucose, ketones, and plasma nonesterified fatty acids to fasting and epinephrine injection in infants and children

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THE varying ability of infants and children to maintain a constant metabolic status in the face of caloric deprivation has stimulated much interest. One of the aspects of this variation which has attracted considerable attention is the extent of the ketone body response under the stress of fasting among children of different age groups.

The clinical impression that very young infants develop ketosis to a lesser degree than older children has been supported by several studies in the past. Gray[1] in 1937 reported low blood ketone concentrations in children under 12 weeks of age with illnesses associated with caloric deprivation due to vomiting. Heymann[2] observed that infants under 7 to 8 months of age developed practically no ketonuria on a ketogenic diet, although infants of similar ages excreted orally administered acetone and sodium acetoacetate in the same quantities as older children. This suggested that the young infants' failure to develop ketonuria was not a reflection of a decreased renal excretion of these substances. Studies on ketone production by young rats have produced conflicting results. Black, Collip, and Thompson[3] observed that 40-day-old rats excreted only minimal amounts of acetone on a ketogenic diet, but a rapid accumulation of blood ketones was noted during starving of 30-day-old rats by Wick and McKay.[4]

It seemed important to restudy this problem in order to establish more precisely the relationship of age to the development of ketosis, and to determine whether the differences were related to production, excretion, or utilization. The relationship of the magnitude of ketosis on fasting to the availability of carbohydrate stored as liver glycogen was also investigated, as was the relationship between fat mobilization and ketosis.

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MATERIALS AND METHODS

A. Measurements were made of the concentrations of ketones, glucose, and plasma nonesterified fatty acids (NEFA) in whole venous blood following fasting in 181 infants and children ranging in age from birth to 12 years (all determinations were not made in every subject). Subjects were selected from the newborn nurseries of the Pennsylvania Lying-In and Methodist-Episcopal Hospitals, the nursery of Clinton Farms, and the wards of The Children's Hospital of Philadelphia. No child was included who was not considered to be metabolically normal. The duration of fasting was 24 hours with the exception of the group of infants who were fasted from birth for periods of 16 to 36 hours (average 27 hours). The infants were given sterile tap water in amounts equivalent to their usual formula and the older children were allowed tap water on demand to prevent dehydration.

B. Epinephrine tolerance tests were performed after a 12 hour fast on 89 subjects ranging in age from birth to 14 years with the use of 0.03 ml. per kilogram of epinephrine 1:1,000 aqueous solution subcutaneously up to a maximum dose of 0.5 ml. Blood was drawn before and 1 hour after injection. Ketone, glucose, and NEFA levels were determined. Similar tests were performed after a 24 hour fast on 28 of the infants, mentioned under A, whose ages ranged from 16 hours to 6 days of age.

C. The rate of renal excretion of ketone bodies was studied in 8 infants 3 to 6 days of age and in 10 children 3½ weeks to 3½ years old following a 24 hour fast. In the older group of children endogenous creatinine and ketone body excretion was studied by standard clearance methods.[5, 6] In the young infants, urine collections were made without catheterization for carefully measured periods of time ranging from 2 to 4½ hours and one blood specimen was drawn at approximately the midpoint of the collection period. Creatinine clearances were calculated and were normal for the age in all subjects studied. Comparisons of ketone excretion were made on the basis of milligrams excreted per hour per square meter of surface area.

D. Utilization of ketone bodies by 9 infants aged 3 days to 15 months was investigated by measurement of blood levels of ketone bodies during continuous infusion of beta-hydroxybutyric acid (BHB). Sodium BHB solutions for intravenous administration were prepared with the use of pyrogen-free water, adjusted to pH 7.0 to 7.4 with 2.0 N HCL and autoclaved for 30 minutes at 12 pounds pressure. A priming injection of 165 mg. per kilogram of NaBHB was administered as a 10 per cent solution, followed immediately by a continuous infusion of 2 per cent NaBHB at the rate of approximately 3 mg. per kilogram per minute. After 30 to 40 minutes was allowed for equilibration, blood was sampled at the beginning and at then end of a 6 to 17 minute period throughout which urine was collected by catheter. The rate of infusion was then increased to a preselected level and after 20 to 30 minutes equilibration blood and urine were again sampled. Whole blood pH and CO_2 determinations during the first

Table I. Frequency of subjects with blood sugar values less than 50 mg. per cent and less than 40 mg. per cent after a 24 hour fast

Age group	No. of subjects	No. of subjects with fasting blood sugar less than		% of subjects with fasting blood sugar less than	
		50 mg.%	40 mg.%	50 mg.%	40 mg.%
16 to 36 hours	59	26	13	44.1	22.0
2 to 6 days	39	30	24	76.9	61.5
1 week to 6 months	25	11	8	44.0	32.0
7 months to 4 years	8	1	1	12.5	12.5
4 to 12 years	10	1	0	10.0	0.0

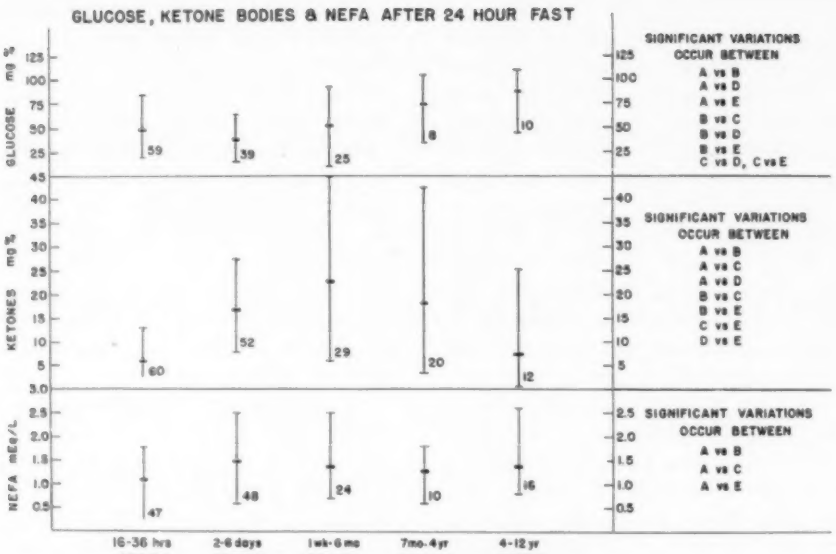

Fig. 1. Blood glucose and ketone bodies and plasma NEFA after a 24 hour fast. The horizontal bars indicate the mean levels for each age group. The vertical lines indicate the range of values within each group. The numbers beside the vertical lines refer to the number of subjects in each group. Age groups between which significant differences exist ($p = < 0.05$) are indicated at the right.

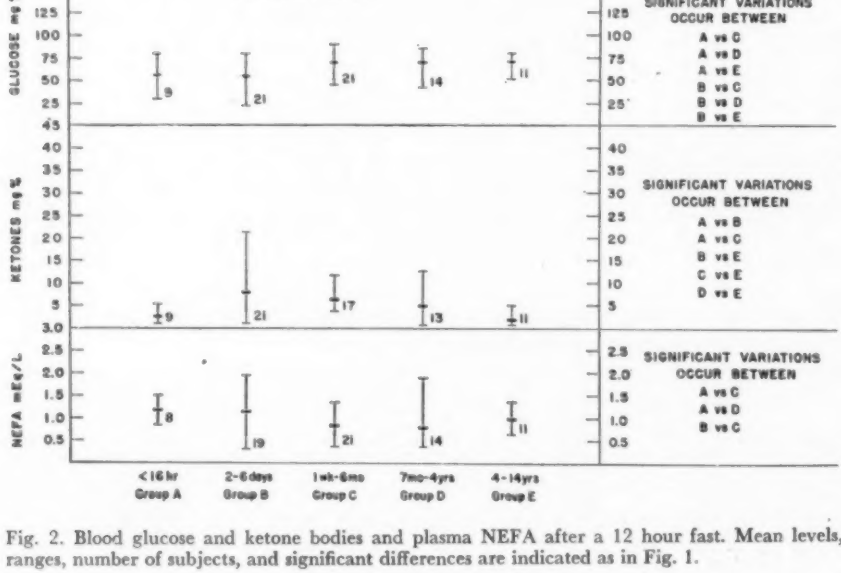

Fig. 2. Blood glucose and ketone bodies and plasma NEFA after a 12 hour fast. Mean levels, ranges, number of subjects, and significant differences are indicated as in Fig. 1.

few infusions indicated no evidence of metabolic alkalosis.

Blood glucose was determined by the Nelson modification of the Somogyi method,[7] and blood ketone levels according to the method of Michaels and co-workers,[8] with the following modifications: The determinations were carried out in ampules rather than bottles and 1 ml. 13.6 N sulfuric acid and 1 ml. 0.4 per cent potassium dichromate were added to the ampules initially, after which the samples were autoclaved for 30 minutes at 15 to 20 pounds pressure. The method of Gordon[9] was used for NEFA determination. All determinations were carried out in duplicate.

RESULTS

The findings in infants and children who had been fasted 24 hours are given in Fig. 1. Group A refers to infants fasted from birth, Group B to infants 2 to 6 days old, Group C to infants 1 week to 6 months, Group D to children 7 months to 4 years, and Group E to children 4 to 12 years.

These data indicated that infants fasted for 16 to 36 hours following birth and children over 4 years developed significantly less ketonemia than did subjects of intermediate age. Contrary to the generally held belief that young infants are resistant to ketosis, infants of 2 to 6 days exhibited a mean blood ketone value of 16.9 mg. per cent with a range of 8.2 to 27.4 mg. per cent. Fasting ketonemia was maximal in the subjects 1 week to 6 months old with a mean of 22.9 mg. per cent and a range of 7.6 to 45.0 mg. per cent, although not significantly greater than in subjects 7 months to 4 years of age (mean 18.4 mg. per cent, range 3.6 to 42.3 mg. per cent).*

Blood glucose determinations in infants fasted 16 to 36 hours following birth averaged 59.9 mg. per cent with a range of 22.2 to 85.9 mg. per cent, suggesting the presence of ample glucose stores at birth in the group as a whole. By 2 to 6 days of age the infants fasted 24 hours revealed a more marked tendency to "hypoglycemia" (mean 38.7 mg. per cent, range 16.0 to 67.6 mg. per cent). This was significantly lower than that of all other age groups. With increasing age (Groups C, D, and E) there was a progressive rise in mean fasting blood sugar concentration toward the adult normoglycemic range. Fasting blood sugar levels below 40.0 mg. per cent were encountered in all age groups except the oldest. The percentage of subjects with blood sugar values less than 50 mg. per cent and less than 40 mg. per cent is shown in Table I. There was no significant correlation between individual glucose and ketone determinations within the various age groups with the exception of the 7 month to 4 year group in which there was a negative correlation ($p = <.05$).

It should be emphasized that in spite of the rather frequent occurrence of low blood sugar levels, all infants and children in the study group tolerated the fast well with no symptoms of hypoglycemia other than hunger. This is in contrast to a 20-month-old male infant with hyperinsulinism who had a convulsion after a 16 hour fast before operation but tolerated a 24 hour fast without difficulty after the removal of two hyperplastic islet tumors, and an 8-month-old female infant with maple syrup urine disease whose only signs of severe hypoglycemia (fasting blood sugar 6 mg. per cent) were tachypnea and a change from flaccidity to rigidity.

Fasting NEFA values were elevated in all groups (means 1.1 to 1.5 mEq. per liter). In all subjects except 2 in the youngest group the values were above those which we had previously determined for young infants in the postprandial period (mean 0.4 mEq. per liter). The mean NEFA value for the subjects 16 to 36 hours old was significantly lower than that of the older subjects in Groups B, C, and E. Observed differences in fasting NEFA levels among the four older groups, B, C, D, and E, were not significant.

*It should be stated that because of the marked skew toward the lower levels in the ketones for the group aged 4 to 12 years, "student's" t test is not correctly applied in this one instance. However, the skew only accentuated the tendency toward low levels in the older age group as indicated by the "t" test. For ease of comparison p values pertaining to these data have been retained.

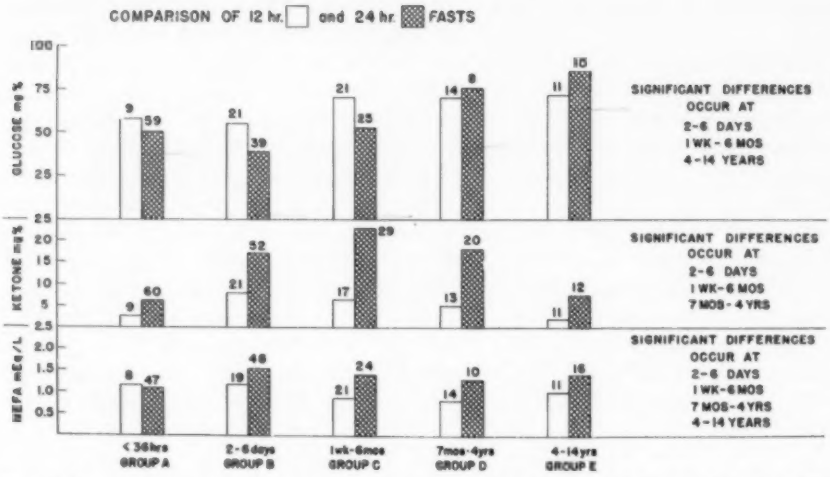
COMPARISON OF 12 hr. and 24 hr. FASTS
GLUCOSE mg %
KETONE mg %
NEFA mEq/L
SIGNIFICANT DIFFERENCES OCCUR AT 2-6 DAYS 1 WK - 6 MOS 4-14 YEARS
SIGNIFICANT DIFFERENCES OCCUR AT 2-6 DAYS 1 WK-6 MOS 7 MOS-4 YRS
SIGNIFICANT DIFFERENCES OCCUR AT 2-6 DAYS 1 WK-6 MOS 7 MOS-4 YRS 4-14 YEARS
< 36 hrs GROUP A
2-6 days GROUP B
1wk-6mos GROUP C
7mos-4yrs GROUP D
4-14 yrs GROUP E

Fig. 3. Comparison by age group of mean values after 12 and 24 hours of fasting. The height of the bars indicates the mean for each group. Clear bars represent the 12 hour fasts and shaded bars the 24 hour fasts. The number of subjects in each group is indicated at the top of each bar. Those age groups in which significant differences occurred are indicated at the right.

There was no evidence of an inverse correlation between plasma NEFA and blood glucose levels in individuals within any one age group.

The 12 hour fasting control values in the 89 individuals subjected to epinephrine tolerance tests were evaluated in a manner similar to those obtained during 24 hour fasting in order to learn how early the differences between the several groups became apparent (Fig. 2). After 12 hours of fasting, the difference in blood glucose concentrations was not yet apparent between the two groups of newborn infants. However, both of these groups had significantly lower glucose levels than the older children and values below 40 mg. per cent occurred only in these two groups. When mean glucose levels after 12 hours of fasting were compared to those of the same age group fasted for 24 hours, significant decreases occurred only in Groups B and C (Fig. 3). Interpreted in terms of rate of fall of blood glucose concentration, this indicates that infants under 36 hours and children over 7 months are able to maintain a fairly constant blood sugar level during the latter half of a 24 hour fast while in infants from 2 days to 6 months of age blood sugar levels are not well maintained in the presence of caloric deprivation.

Infants fasted 12 hours following birth exhibited significantly lower ketone concentrations than infants of 2 days to 6 months. Of all the groups studied, infants 2 to 6 days of age had the highest ketone concentrations after 12 hours of fasting, suggesting an earlier onset of ketosis. In spite of the ability of children 7 months to 4 years of age to maintain a fairly stable glucose level during a 24 hour fast, ketone bodies accumulated fairly rapidly during the last 12 hours of the 24 hour fast.

With the exception of a few individuals in Groups B and D, 12 hours of fasting represented sufficient stress to elevate NEFA concentrations above the postprandial level of 0.4 mEq. per liter. When 12 and 24 hour fasts were compared, there was no significant rise in NEFA in infants fasted from birth but the older children showed a consistent further increase of 0.4 to 0.5 mEq. per liter.

When a group of infants fasted following

birth for 36 to 48 hours were compared with the less severely stressed newborn infants discussed above, the glucose concentrations were seen to fall only slightly over the 48 hour period from a mean of 58 mg. per cent under 16 hours to 51 mg. per cent at 16 to 36 hours to 43 mg. per cent for infants 36 to 48 hours old. Ketone body concentrations for the same intervals showed a steady progression from 2.4 mg. to 6.4 to 12.3 mg. per cent, respectively. There was a strong positive correlation between length of fast and blood ketone levels for which $p = < .01$. This indicated that even during the first 2 days of life the capacity to produce ketone bodies is not lacking. NEFA levels determined at the above intervals in this age group did not rise progressively with the length of the fast.

These findings of considerable metabolic stability during the first 24 to 48 hours of life suggest that very young infants have a substantial supply of available carbohydrates, most likely in the form of liver glycogen, which serves to carry them safely through the first day or 2 of life when caloric intake is usually low. To test this reserve and its apparent depletion during the 2 to 6 day period, epinephrine tolerance tests were performed on 9 infants less than 16 hours of age and 13 infants 16 to 36 hours of age. In the 2- to 6-day-old group, 21 infants were studied after a 12 hour fast and 15 infants after a 24 hour fast. The older age groups, C, D, and E, were also studied in this manner after a 12 hour fast for purposes of comparison.

The results of these studies are shown in Fig. 4. It was demonstrated that the absolute increase in glucose concentration after epinephrine was significantly higher in infants under 16 hours of age fasted from birth as compared with that of 2- to 6-day-old infants fasted 12 hours (63.1 as against 37.3 mg. per cent, $p = < .001$). This difference in response to epinephrine persisted when the test was performed after a 24 hour fast (68.6 as against 37.1 mg. per cent, $p = < .01$) and with no significant alteration in the degree of response after 12 contrasted with 24 hours of fasting. Since the length of fast did not appear to affect the

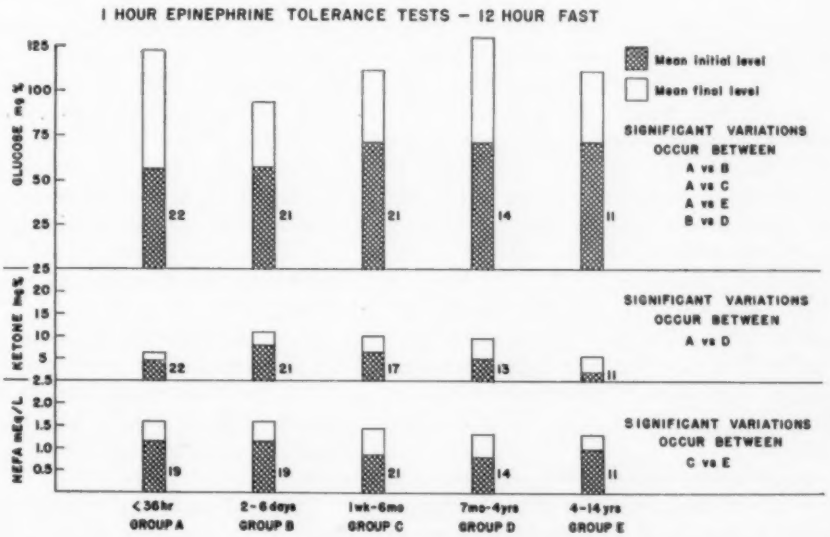

Fig. 4. Epinephrine tolerance tests. The heights of the shaded bars indicate the mean fasting values before epinephrine and the clear areas the response to epinephrine. The numbers beside the bars indicate the number of subjects in each age group. Significant differences in response are indicated at the right.

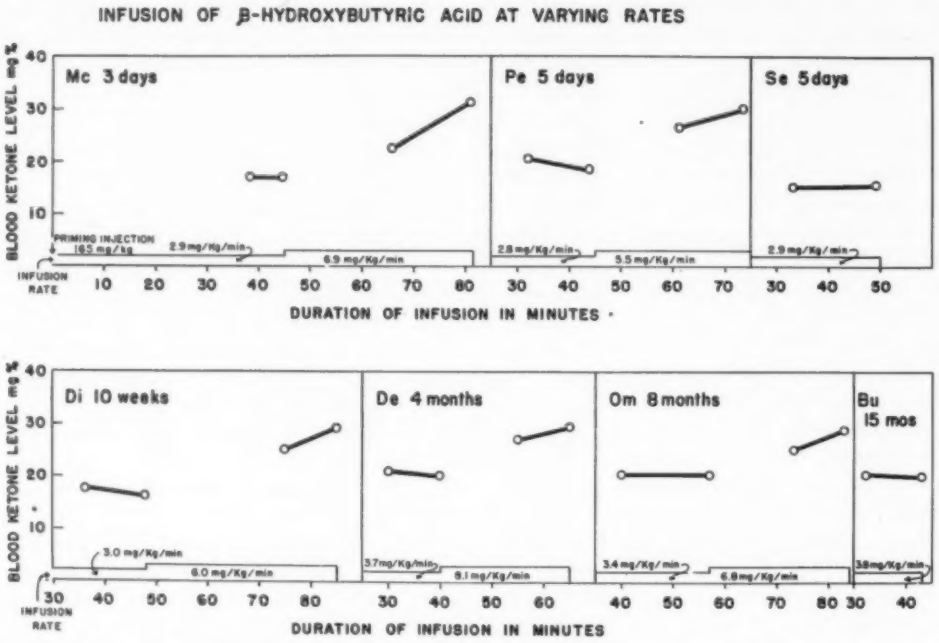

Fig. 5. Infusion of beta-hydroxybutyric acid at varying rates to determine approximate rates of utilization. At the bottom of each figure are given the rate and duration of the experimental infusions. A prior priming injection of 165 mg. per kilogram was given to each of the patients but is represented only at the left-hand bottom corner of the first figure, Patient Mc. Solid lines indicate the test periods during which urine was collected.

response to epinephrine in the infants under 36 hours of age, they are treated as a single group in later discussions and in Fig. 4.

In the two younger age groups the difference in ketone body response was not quite as striking; however, 68 per cent of the infants under 36 hours of age had ketone increases of less than 2.0 mg. per cent as compared with only 19 per cent of the infants 2 to 6 days old.

In Groups C, D, and E, glucose and ketone body responses to epinephrine were similar. The mean increase in glucose values ranged from 40 to 55 mg. per cent, and in ketone bodies ranged from 3.6 to 6.6 mg. per cent.

After epinephrine, NEFA rose between 0.5 and 0.6 mEq. per liter in all age groups under 4 years, indicating that the failure of infants under 36 hours of age to develop ketonemia and elevated NEFA on fasting does not represent lack of ability to mobilize depot fat, in response to epinephrine stimulation.

The NEFA levels obtained after epinephrine were as high as those following a 24 hour fast in all age groups, except in the infants under 36 hours of age. The ketone levels found after epinephrine were definitely lower than those obtained after a 24 hour fast in all age groups except the youngest in whom the response to epinephrine and fasting were identical.

Because of the possibility that a traumatic venipuncture might produce an epinephrine-like effect[10] the findings in 31 infants were analyzed in relation to this factor and no suggestion of such an effect could be detected.

In the infants and children aged 1 week to 4 years who developed maximal ketosis, consideration of the role played by excretion and utilization became important. No correlation was found between age and ability

to excrete ketone bodies. As can be seen from Table II, the rate of excretion appears to be related to the blood concentrations; there were very high excretions in 2 patients at blood levels of 41 and 45 mg. per cent, respectively, a point at which the renal threshold of 20 mg. per cent for BHB is clearly exceeded.[11] All but one (McL) of the 8 newborn infants had excretions comparable to those of the older children. There was no apparent explanation for the abnormally high excretion of this one infant.

Continuous infusions of BHB were undertaken in 7 subjects to determine if there were any differences in rate of ketone body utilization in relation to age (Fig. 5). The assumption was made that a constant blood level indicated the rate of infusion to be equal to the rate of utilization while a rising blood level indicated that the rate of utilization had been exceeded.

Infusion rates between 2.8 and 3.8 mg. per kilogram per minute resulted in constant or slightly falling blood ketone levels. This suggests that rates of infusion of BHB of approximately 3 mg. per kilogram per minute were equal to or somewhat less than the subject's capacity for utilization. Increasing the rate of infusion to 5.5 to 8.1 mg. per kilogram per minute in 5 of these patients apparently exceeded this capacity and resulted in a rise in blood concentration. From these data it would appear that the capacity for utilization of exogenous BHB is about 3 to 5 mg. per kilogram per minute, and is independent of the age of the subject within the range of 3 days to 15 months. This value is of similar magnitude to the mean rate of ketone body utilization which Stadie[12] reported to be 1.7 mg. per kilogram per minute in aketonuric adults, with gradually increasing rates in the presence of ketonuria.

The possibility was considered that the rise in concentration noted at the higher rate of infusion was related to the duration of the infusion and would have occurred in time at the lower rate. For this reason two infusions were given at constant rates over 90 minute periods. Fig 6 shows that the subject who received BHB at the rate of 5.2 mg. per kilogram per minute maintained a nearly constant blood level throughout the entire period, while the other subject, receiving 6.9 mg. per kilogram per minute, showed

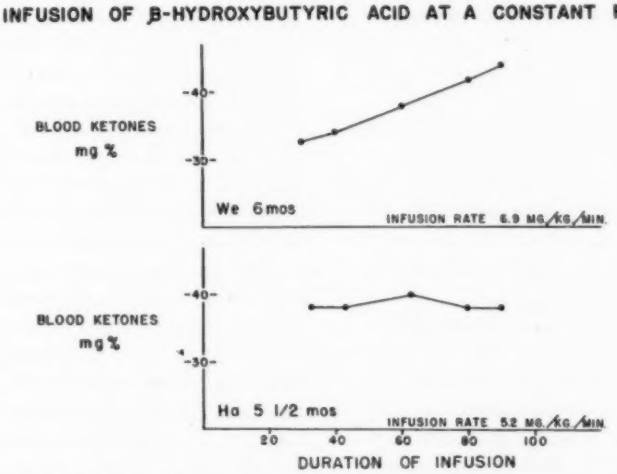

Fig. 6. Infusion of beta-hydroxybutyric acid at two rates to determine the effect of a prolonged infusion. A priming injection of 165 mg. per kilogram of BHB was given at the beginning of each infusion at zero time. Rate and duration of infusion are indicated at the bottom of each figure.

Table II. Urinary excretion of ketone bodies

Subject	*Age*	*Creatinine clearance (ml./min../1.73 M²)*	*Blood ketone concentration (mg.%)*	*Ketone excreted (mg./hr./M²)*
2- to 6-day-old infants, voided urine specimens				
McL	4 days, 2½ hours	17.9	13.6	37.8
Sco	3 days, 15½ hours	32.6	24.8	18.4
Wil	3 days, 6 hours	26.2	17.4	11.0
Bos	5 days, 2 hours	20.7	23.5	17.3
Ped	5 days, 17½ hours	53.8	27.4	32.6
Cra	5 days, 1 hour	31.2	12.0	11.0
Pac	3 days, 16 hours	20.9	15.1	7.0
Bar	3 days 6½ hours	26.5	20.0	17.6
3-week to 3-year-old subjects, catheterized urine specimens				
			Initial-final	
Le	3⅓ weeks	88.0	5.2- 6.8	5.5
Mo	6 weeks	75.5	7.0- 6.6	8.2
Sp	10 weeks	74.5	9.7-14.3	8.6
Sc	10 weeks	91.9	18.2-20.8	11.1
Si	6 months	80.1	19.8-25.4	8.3
Ro	8 months	82.2	8.8-11.1	4.5
Mu	10 months	59.9	42.3-47.4	96.0
Da	15 months	78.1	25.5-26.6	7.5
Lo	15 months	81.0	40.2-41.4	49.5
Bl	3⅓ years	91.9	3.4- 4.0	5.5

a rising blood concentration after 30 minutes.

The amount of administered BHB lost by urinary excretion was measured for each of the time intervals involved and was found to be between 0.2 and 8.1 per cent (mean 2.9 per cent) of the total amount given, indicating that the main function tested was that of utilization. These studies also showed no trend of excretion in relation to age.

DISCUSSION

The finding that infants and children of all ages are capable of developing a significant degree of ketosis after the stress of a 24 hour fast with no further marked increase in the degree of ketosis at or around 3 months of age is in contrast to the findings of earlier workers who had concluded that very young infants are unusually resistant to ketosis.

The discrepancy in findings may be the result of differences in experimental conditions both in nature and degree of the ketogenic stimulus used in the various studies. Gray's[1] patients were not completely fasted but appear to have been on suboptimal caloric intakes because of vomiting. In Heymann's[2] studies ketogenic diets were employed and it was shown that infants under 7 months of age developed very little ketonuria. His results were similar to the findings of Black and associates[3] in 40-day-old rats on ketogenic diets but in contrast to those of Wick and McKay[4] in fasted 30-day-old rats which developed elevated blood ketone levels in a fashion similar to the infants in the present study.

It is inappropriate to compare studies of animal and human responses to ketogenic diets with those based on fasting. Metabolic adaptations to high fat diets occur which enable the organism to burn fatty acids prior to carbohydrate depletion and to withstand fasting with less ketosis, glycogen depletion, and loss of ability to synthesize fatty acids.[13]

Clinical observations of the rarity of acetonuria in very young as compared to older infants with severe diarrhea and vomiting may not be valid for two reasons. First, many of these infants do not void until after having received significant quantities of glucose intravenously or orally and may therefore have metabolized their accumulated ketone bodies by the time a urine specimen

is obtained. Second, residual dehydration, in very young infants, may play a role in this phenomenon in view of the work of Johnson and Passmore,[14] who have reported that post-exercise ketosis is diminished in the presence of dehydration. Differences in time of voiding and degree of dehydration in these age groups have not been documented but both factors may play a role in contributing to the observed difference.

The relative ability of infants under 36 hours of age to resist fasting ketosis is apparently due to the presence of stores of liver glycogen which provide sufficient available carbohydrate to prevent ketone body accumulation. This interpretation is supported by the ability of these infants to maintain a rleatively high blood glucose and low plasma NEFA even after extension of the fast to 48 hours after birth and to release large quantities of glucose into the blood stream following epinephrine injection. Since epinephrine does produce an elevation of NEFA and ketone bodies in these subjects and since the ketone level steadily rises during the 48 hour fast, there is no evidence of a lack of ability to catabolize fat or to produce ketone bodies. In the presence of a steady flow of carbohydrate from the liver, the magnitude of fat breakdown during the first day of life is not sufficient to elevate ketone bodies to any great extent. Other evidence to support this conclusion comes from the finding of Benedict and Talbot[15] that the respiratory quotient shortly after birth ranges from 1.0 to 0.9, indicating carbohydrate as the chief source of energy. In contrast to this, the respiratory quotient of infants 2 to 6 days of age was found to be between 0.7 and 0.8, which suggests subsistence chiefly on fat and correlates with our findings of low blood glucose, high blood ketone, and elevated NEFA levels in this group. Even after resuming feeding for 2 days, these infants did not become completely free of ketosis as was seen in 4 subjects whose ketone levels were 5 to 8 mg. per cent 3 hours after their last feeding. This further suggests depletion of the carbohydrate stores at this age even when supplemented by the carbohydrate of the diet. Autopsies on infants who died at or shortly after birth have also shown the presence of large amounts of liver glycogen, while the livers of infants who came to autopsy at a few days of age have shown a reduced glycogen content similar to that of the adult.[16]

Studies measuring excretion and utilization of ketones in the 3 day to 4 year groups failed to explain the high average fasting ketone levels or the differences between individuals or age groups. With one exception, excretion studies in subjects whose ages ranged from 3 days to 3½ years and whose fasting ketone levels ranged from 3.4 to 47.4 mg. per cent revealed approximately the same rate of excretion which was dependent directly upon blood ketone body concentration and not upon age. Since the rate of utilization seems to be similar in the group of children 3 days to 15 months of age who fall in the age group which becomes especially ketotic on fasting and in adults[12] who are relatively resistant to ketosis, it appears that the factors producing the age-related differences in ketosis following fasting are not likely to be related to utilization or to excretion but rather to the rate of ketone body production.

Although the relatively low levels of ketone bodies found in infants fasted up to 36 hours after birth seem to be clearly related to a supply of liver glycogen adequate for their needs, and the high levels in 2- to 6-day-old infants appear to be secondary to depleted carbohydrate reserves, the factors responsible for producing differences among the older groups are not clear. In the children over 4 years of age the more effective maintenance of normal blood sugar concentrations and lesser degree of ketosis may be manifestations of increased adrenal cortical gluconeogenic, glucostatic, and lipogenic effects. Another factor which may contribute to a greater degree of fasting ketosis between 1 week and 4 years is the relatively higher metabolic rate of these children which would lead to a greater demand on fat stores in order to satisfy energy requirements. A clinical application of these findings is that ke-

tosis may contribute to metabolic acidosis in the presence of caloric deprivation in all age groups but is more apt to reach significant proportions relatively early in patients between the ages of 2 days and 4 years.

Fasting blood sugar levels below 40 mg. per cent have generally been considered of pathologic significance.[17] The frequent finding of blood sugar levels in this range in asymptomatic, fasted children below the age of 4 years indicates that this is not always the case and suggests that caution be observed in attributing a patient's symptoms to hypoglycemia. The present study confirmed the well-known observation that asymptomatic newborn infants may exhibit blood sugar levels as low as 16 to 20 mg. per cent.[18] Inability to tolerate a 24 hour fast at any age appears to be a definite indication of metabolic abnormality.

In this study, the finding of elevated NEFA following fasting and epinephrine injection parallels that of Dole,[19] Gordon and Cherkes,[20] and Havel and Goldfien.[21] From Gordon's studies[9] of NEFA arteriovenous differences in fasting subjects and from the work on epinephrine effects on isolated fat tissue by Reshef and Shapiro[22] it is assumed that the changes in NEFA levels observed may be attributed to mobilization of fatty acids from fat depots to other tissues for metabolism[23, 24] as the availability of glucose to the tissues becomes diminished during fasting or to activation of lipolytic enzymes under the influence of epinephrine. Since NEFA levels do not increase in proportion to ketone elevation, increased concentration of NEFA indicates fat mobilization but is not a sensitive reflection of the extent of this process. For example, Groups B, C, and D with the highest ketone elevations showed no greater NEFA rise than did Group E in which ketone increase was slight. However, there was a small increase in NEFA elevation after 24 hours of fasting over the 12 hour fasting levels, indicating some degree of correlation with increasing dependency on fat utilization for energy requirements. This change was of lesser magnitude than the relative increase observed in ketone levels over the same period of 12 hours in subjects between 1 week and 4 years of age. This difference might be expected in view of the rapid utilization of NEFA as shown by its short half life[25] and its major role in satisfying the energy requirements of the fasting organism.

In conclusion, these data indicate that infants under 36 hours and children over 4 years of age show an increased ability to resist ketosis in the face of caloric deprivation as compared with subjects in intermediate age groups.

SUMMARY

1. Blood ketone, blood glucose, and plasma NEFA concentrations after a 24 hour fast were studied in 181 infants and children from birth to 12 years of age.

2. The same measurements following epinephrine injections were made in 89 infants and children from birth to 14 years.

3. It was found that infants under 36 hours were more resistant to ketosis on fasting than infants 2 to 6 days of age whose ketosis became almost as severe as that of subjects aged 1 week to 4 years. The difference in response between these two newborn groups was found to be associated with large stores of liver glycogen in the younger group. Infants under 36 hours did not appear to have any impairment of fat catabolism as indicated by a progressive rise in blood ketone levels on more extended fasts and by their normal NEFA elevation after epinephrine.

4. Subjects 1 week to 4 years of age developed more ketosis on fasting than did older children. It was shown that this was not related to differences in excretion or utilization of ketone bodies, indicating that variation in ketone production was the factor responsible.

5. Blood sugar levels below 40 mg. per cent occurred in 45 of 123 subjects under 6 months of age who were fasted for 24 hours. With one exception, blood sugar below this level was not found in subjects over 7 months of age.

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Renal excretion of hydrogen ions: A defense against acidosis in premature infants

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THE existence of metabolic acidosis in premature infants has been confirmed by many observers.[1-3] In some instances, respiratory acidosis has also been present along with the metabolic acidosis. To a lesser degree, metabolic acidosis has been noted to occur in full-term infants in the early newborn period.[4-7]

The current concept of renal regulation of acid-base balance[8] would indicate that the essential mechanism in renal control of base conservation is the hydrogen-sodium exchange in the renal tubules. Through this mechanism, bicarbonate reabsorption, the excretion of ammonium salts, and the acidification of urinary buffers are accomplished. Tudvad, McNamara, and Barnett[9] have demonstrated that the healthy premature infant is capable of reabsorbing bicarbonate at a rate comparable to that of the adult. The studies described below were designed to test the 2 other mechanisms of renal regulation of acid-base metabolism in premature infants. Gordon, McNamara, and Benjamin[10] in a study of 3 premature infants given an ammonium chloride load showed that 2 of their infants had a diminished ammonium excretion (with increased sodium losses) when compared with full-term infants treated in the same manner; however, there was essentially no associated increase in the titratable acid excretion. It would appear from these data that there is also a deficiency in the hydrogen-sodium ion exchange mechanism involving the urinary buffers in some premature infants, which could account for a tendency toward metabolic acidosis.

In this report, studies will be presented to explore further the renal capacity of premature infants to excrete ammonium salts and to acidify urinary buffers.

METHODS AND PROCEDURE

The subjects of this study were 9 healthy prematurely born infants, 26 to 49 days of age and weighing 1,400 to 2,640 grams at the onset of the study period. These infants were divided into 2 groups, the smaller infants weighing approximately 1.5 kilograms

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(26 to 28 days of age) and the larger infants weighing approximately 2.5 kilograms (43 to 49 days of age). These infants were given approximately 10.7 mEq. of calcium chloride orally per kilogram of body weight per day (range—7 to 14.4 mEq.) in 4 divided doses, incorporated in the formula for 3 to 21 days. All infants were fed Alacta formula at a caloric intake of 150 calories per kilogram per day. A neutral sodium phosphate (pH 7.4) 0.15 molar solution was administered intravenously over a period of 30 minutes to 2 infants at the termination of the oral ingestion of calcium chloride given for 10 days and 3 days, respectively; one received 85.5 mg. per kilogram and the other 189 mg. per kilogram.

Urine specimens were obtained daily under oil with thymol crystals as a preservative. The collection flask was immersed in ice during the collection. Urine was obtained by catheterization. The urine samples were analyzed in duplicate for pH, chloride, ammonia, sodium, potassium, titratable acid, organic acids, and phosphates. Blood samples were obtained from the femoral vein and immediately immersed under oil for determination of: carbon dioxide content, chloride, sodium, calcium, and phosphate. Serum carbon dioxide content was determined by the method of Van Slyke.[11] Glass electrode pH analysis was used for urinary pH estimations. Chloride was determined by the method of Sendray as modified by Van Slyke and Hiller,[12] and ammonia by the microdiffusion method of Conway.[13] Sodium and potassium were analyzed using the Barclay flame photometer.[14] Phosphorus was determined according to the method of Gomori[15] and titratable acids according to that of Henderson and Palmer.[16] Organic acids were determined by the method of Van Slyke and Palmer,[17] and serum calcium by the method of Roe and Kahn.[18] No infant in this study became ill or showed any adverse effects during or following the study.

RESULTS

Table I lists the data obtained before, during, and following the administration of calcium chloride to the smaller premature infants with an average weight of 1.5 kilograms. In all instances, acidosis was induced, resulting in a serum CO_2 content below 20 mEq. per liter. In 3 of the 4 premature infants, there was a rise in serum chloride concentration.

Table II shows similar data obtained in larger premature infants with an average weight of 2.5 kilograms.

It is not clear whether the capacity for ammonium excretion should be compared to the adult on a basis of body weight or surface area, therefore, the absolute values are listed in Tables I and II.

Effects of calcium chloride loading. Urinary chloride excretion increased from a base line average of 2.2 mEq. per kilogram per day and rose to an average of 6.4 mEq. per kilogram per day in the smaller infants. In the older infants, the value changed from an average of 2.1 mEq. per kilogram per day to 6.3 mEq. per kilogram per day. The urinary ammonia excretions in the smaller infants rose from an average of 0.60 mEq. per kilogram per day to 2.7 mEq. per kilogram per day, and in the larger infants, the rise was from 1.12 mEq. per kilogram per day to 4.12 mEq. per kilogram per day. Urinary titratable acid excretions rose only slightly

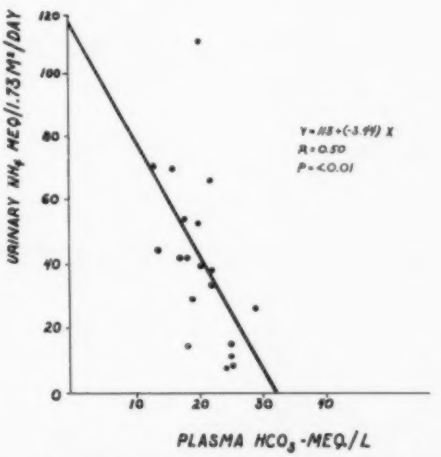

Fig. 1. The relationship between urinary ammonium excretion and plasma CO_2 content concentration in the smaller premature infants.

Table I. Smaller premature infants during calcium chloride administration

	Day	Serum CO_2 (mEq./L.)	Serum Na (mEq./L.)	Serum Cl (mEq./L.)	$CaCl_2$ intake (mEq.)	24-hour urine Vol. (c.c.)	pH	Cl (mEq.)	NH_3 (mEq.)	Na (mEq.)	K (mEq.)	T.A. (mEq.)	O.A. (mEq.)	PO_4 (mg.)
B. B., aged 27 days, weight 1.43 kilograms, surface area 0.1409 M.²														
Base line	1	25.7	143	103	–	115	6.78	5.28	0.95	4.62	5.54	0.78	2.47	1.58
	2	–	–	–	–	103	6.55	2.74	1.02	2.06	4.34	1.03	2.48	1.48
	3	25.7	145	104	–	93	6.61	2.37	0.66	1.65	3.85	0.77	–	1.40
Total		51.4	288	207	–	311	19.94	10.39	2.63	8.33	13.73	2.58	4.95	4.46
Average		25.7	144	104	–	104	6.65	3.46	0.88	2.78	4.58	0.86	2.40	2.48
$CaCl_2$ loading period	4	–	–	–	14.3	96	6.48	5.47	1.54	3.18	5.19	0.92	2.09	1.31
	5	–	–	–	14.3	118	5.59	9.24	2.01	4.38	4.96	1.07	2.04	0.72
	6	19.6	–	–	14.3	128	5.51	9.32	2.43	5.00	4.88	1.28	2.04	0.86
	7	–	–	–	14.3	100	5.70	8.74	2.61	4.10	3.32	0.60	1.34	0.28
	8	–	–	–	14.3	130	5.92	8.91	2.42	3.91	4.30	0.78	1.86	0.53
	9	22.4	–	103	14.3	130	5.59	10.48	3.12	3.91	5.10	1.04	2.52	0.79
	10	–	–	–	14.3	121	5.80	9.85	2.78	2.79	5.84	0.99	2.58	0.90
	11	–	–	–	14.3	137	6.03	11.98	3.57	4.12	5.65	0.78	2.60	0.75
	12	21.2	154	100	14.3	104	6.26	8.92	3.22	2.71	4.81	0.48	2.43	0.77
Total		63.2	154	203	128.7	1064	52.88	82.91	23.70	34.10	44.05	7.94	19.50	6.91
Average		21.1	154	102	14.3	118	5.88	9.21	2.63	3.79	4.89	0.88	2.17	0.77
Recovery	13	–	–	–	–	76	6.25	2.92	1.82	0.70	2.22	0.53	1.47	0.73
	14	–	–	–	–	76	5.97	2.93	2.28	0.53	3.44	1.13	1.95	1.55
	15	–	–	–	–	92	6.16	3.62	2.30	0.63	5.06	1.30	2.28	2.00
	16	29.4	135	95	–	92	6.23	3.58	2.22	1.01	5.52	1.49	2.26	2.68
	17	–	–	–	–	126	6.36	2.65	2.39	1.39	5.93	1.46	3.10	2.84
Total		29.4	135	95	–	462	30.97	15.70	11.01	4.26	22.17	5.91	11.06	9.80
Average		29.4	135	95	–	92	6.19	3.14	2.20	0.85	4.43	1.18	2.21	1.96
B. W., aged 28 days, weight 1.40 kilograms, surface area 0.1591 M.²														
Base line	1	–	–	–	–	132	5.28	4.07	1.57	2.68	6.68	3.34	2.75	2.76
	2	–	–	–	–	132	5.16	3.99	1.08	2.38	6.63	3.65	2.85	3.01
	3	18.7	137	99	–	118	5.49	3.63	1.36	1.99	6.34	3.04	2.78	2.88
Total		18.7	137	99	–	382	15.93	11.69	4.01	7.05	19.65	10.03	8.38	8.65
Average		18.7	137	99	–	127	5.31	3.90	1.34	2.35	6.55	3.34	2.79	2.88

$CaCl_2$	4	–	–	–	17.9	140	5.30	8.50	3.16	4.12	8.31	3.04	3.96	2.96
loading	5	–	–	–	17.9	204	5.88	10.89	4.50	4.07	4.51	2.46	2.58	2.48
period	6	20.1	137	107	17.9	140	6.38	11.38	10.20	6.32	7.02	2.00	6.04	2.51
	7	–	–	–	17.9	178	6.02	11.51	12.55	6.60	8.57	2.82	5.00	3.20
	8	–	–	–	17.9	190	6.48	11.68	7.62	7.62	6.87	2.59	3.71	4.01
	9	16.0	–	105	17.9	184	6.28	12.05	6.44	7.20	6.65	1.92	2.62	3.18
	10	–	–	–	17.9	142	5.56	9.01	4.27	3.71	3.71	2.45	2.44	2.76
	11	–	–	–	17.9	130	5.88	8.35	4.75	3.38	4.75	2.31	2.52	2.94
	12	13.1	131	113	17.9	156	5.91	12.31	6.70	5.12	5.72	2.55	3.47	3.30
Total		49.2	268	325	161.1	1464	53.69	95.68	60.19	48.14	56.11	22.14	32.34	27.34
Average		16.4	134	108	17.9	163	5.97	10.63	6.69	5.35	6.23	2.46	3.59	3.04
Recovery	13	–	–	–	–	167	7.55	10.82	–	4.02	5.03	–	5.18	3.01
	14	–	–	–	–	166	7.00	7.02	–	2.50	4.16	–	4.09	3.76
	15	–	–	–	–	176	6.18	5.72	6.54	2.12	4.95	1.47	3.55	3.82
	16	22.0	134	104	–	159	6.54	4.33	6.22	1.02	5.10	0.00	1.81	3.35
	17	–	–	–	–	156	5.51	4.17	4.54	1.12	6.26	3.85	2.97	4.96
Total		22.0	134	104	–	824	32.78	32.06	17.30	10.78	25.50	5.32	17.60	18.90
Average		22.0	134	104	–	165	6.56	6.41	5.77	2.16	5.10	1.77	3.52	3.78

G. F., aged 26 days, weight 1.64 kilograms, surface area 0.1574 M.²

Base line	1	–	–	–	–	90	6.10	3.78	0.69	2.86	4.37	1.39	2.40	1.72
	2	–	–	–	–	–	5.40	–	–	–	–	–	–	–
	3	25.5	135	104	–	65	6.47	2.32	1.46	1.52	3.04	0.78	–	1.02
Total		25.5	135	104	–	155	17.97	6.10	2.15	4.38	7.41	2.17	2.40	2.74
Average		25.5	135	104	–	76	5.99	3.05	1.08	2.19	3.71	1.09	2.40	1.37
$CaCl_2$	4	–	–	–	17.9	116	6.65	7.72	4.72	3.90	–	2.07	4.58	2.97
loading	5	–	–	–	17.9	–	–	–	–	–	–	–	–	–
period	6	18.7	139	109	17.9	145	5.31	8.71	3.89	4.60	6.33	2.97	3.38	2.76
	7	–	–	–	17.9	100	5.32	7.90	1.52	3.75	4.54	1.19	1.60	0.99
	8	–	–	–	17.9	178	6.00	10.56	3.89	4.41	6.71	2.40	3.12	1.43
	9	17.6	133	108	17.9	–	–	–	–	–	–	–	–	–
	10	–	–	–	17.9	–	–	–	–	–	–	–	–	–
	11	–	–	–	17.9	134	6.03	8.42	4.26	3.59	4.79	1.62	3.00	3.52
	12	12.0	147	116	17.9	–	–	–	–	–	–	–	–	–
Total		48.3	419	333	161.1	673	29.31	43.31	18.28	20.25	22.37	10.25	15.68	11.67
Average		16.1	140	111	17.9	135	5.86	8.66	3.66	4.05	5.59	2.05	3.14	2.33

Table I. Cont'd

	Day	Serum			$CaCl_2$ intake (mEq./L.)	24-hour urine								
		CO_2 (mEq./L.)	Na (mEq./L.)	Cl (mEq./L.)		Vol. (c.c.)	pH	Cl (mEq.)	NH_3 (mEq.)	Na (mEq.)	K (mEq.)	T.A. (mEq.)	O.A. (mEq.)	PO_4 (mg.)
Recovery	13	–	–	–	–	172	6.16	9.82	5.13	3.38	5.46	1.94	4.28	2.91
	14	–	–	–	–	178	6.30	7.73	7.59	2.83	5.30	1.73	4.32	4.45
	15	–	–	–	–	160	6.52	5.81	7.61	1.43	4.91	1.06	4.71	3.84
	16	18.0	133	100	–	132	6.54	4.39	4.98	0.78	4.71	1.02	3.84	2.64
	17	–	–	–	–	172	6.42	4.32	5.12	0.68	6.14	1.74	4.44	3.48
Total		18.0	133	100	–	814	31.94	32.07	30.43	9.10	26.52	7.49	21.59	17.32
Average		18.0	133	100	–	163	6.39	6.41	6.09	1.82	5.30	1.50	4.32	3.46
G. M., aged 27 days, weight 1.7 kilograms, surface area 0.1595 M.²														
Base line	1	–	–	–	–	65	6.63	1.87	0.37	1.43	3.29	0.52	–	–
	2	–	–	–	–	77	6.20	2.29	0.62	1.69	3.22	1.11	1.61	1.16
	3	24.9	–	–	–	79	5.74	3.75	0.77	1.84	3.19	1.46	1.49	1.38
Total		–	–	–	–	221	18.57	7.91	1.76	4.96	9.70	3.09	3.10	2.54
Average		24.9	–	–	–	74	6.19	2.64	0.59	1.65	3.23	1.03	1.55	1.27
$CaCl_2$ loading period	4	–	–	–	17.9	144	5.30	8.67	1.58	5.63	6.00	2.15	1.73	1.47
	5	–	–	–	17.9	–	5.08	–	–	–	–	–	–	–
	6	19.7	135	109	17.9	–	4.81	–	–	–	–	–	–	–
	7	–	–	–	17.9	132	5.72	12.36	3.75	6.70	7.47	1.97	3.51	1.75
	8	–	–	–	17.9	215	5.90	12.18	3.86	4.92	9.18	1.98	4.02	1.55
	9	19.9	–	120	17.9	138	5.76	12.45	4.95	3.97	10.83	2.92	5.20	3.00
	10	–	–	–	17.9	114	5.97	10.15	2.95	3.86	8.97	1.63	–	2.14
	11	–	–	–	17.9	120	6.39	9.17	2.87	4.18		0.72	–	1.20
	12	14.1	134	105	17.9	196	6.19	10.07	4.11	5.07	6.84	1.13	2.64	1.02
Total		53.7	269	334	161.1	1059	51.12	75.05	24.07	34.33	54.55	12.50	17.10	12.13
Average		17.9	135	111	17.9	151	5.68	10.69	3.44	4.90	7.79	1.79	3.42	1.73
Recovery	13	–	–	–	–	178	6.22	10.15		3.01	6.92	1.52	–	1.72
	14	–	–	–	–	201	6.19	7.32	4.80	1.96	7.82	2.04	–	2.99
	15	–	–	–	–	155	6.50	3.12	3.56	1.09	5.87	1.39	–	2.36
	16	22.6	–	99	–	164	6.69	1.89	3.11	0.72	5.06	0.95	4.02	2.08
	17	–	–	–	–	164	6.71	3.82	3.11	1.76	8.01	1.17	3.98	2.73
Total		22.6	–	99	–	862	32.31	26.30	19.74	8.54	33.68	7.07	8.00	11.88
Average		22.6	–	99	–	172	6.46	5.26	3.95	1.71	6.74	1.41	4.00	2.38

in both groups. Despite the sharp drop in the serum CO_2 content values in these small infants, the urine pH decreased slightly in only 2 of the 4 smaller premature infants, and there was essentially no change in the remaining 2. The average urinary phosphorus excretion of 1.4 mEq. per kilogram per day did not change in the smaller infants. The urinary sodium excretion rose from an average of 1.5 mEq. per kilogram per day to 3.0 mEq. per kilogram per day in the smaller premature infants and from an average of 1.5 mEq. per kilogram per day to an average of 2.8 mEq. per kilogram per day in the larger infants. The urinary potassium excretions were inconsistent, with an average rise in the smaller infants of 0.92 mEq. per kilogram per day; there was essentially no change in the urinary potassium excretion in the larger infants.

Recovery period following calcium chloride loading in the smaller infants. Urinary ammonia excretions continued at an elevated level and even rose further in 2 of the 4 smaller premature infants when the urinary chloride excretion promptly decreased toward the baseline levels. Titratable acid excretions decreased at a time when urinary phosphorus excretions increased slightly. Urinary sodium excretions decreased below the baseline values. There was a slight decrease in urinary potassium excretion from the values found during the calcium chloride loading period.

Table III lists the data obtained in 2 premature infants rendered acidotic with calcium chloride and then given an intravenous load of isotonic, neutral sodium phosphate. Urinary titratable acid excretion in infant G. F. increased from a control value of 29.0 to 200.0 μEq. per 1.73 M.^{2} per minute during the administration of sodium phosphate. This sevenfold increase in titratable acid excretion is associated with an increase in phosphate excretion from 19.0 to 330.0 μEq. per 1.73 M.^{2} per minute. Serum phosphorus concentration rose from 6.4 to 35.7 mg. per cent, while serum calcium fell from 10.4 to 7.0 mg. per cent during the infusion and to 5.7 mg. per cent during the postinfusion period. The administration of a smaller amount of sodium phosphate to the second infant, G. M., resulted in a rise in serum phosphorus concentration from 8.8 mg. per cent to 10.8 mg. per cent. There was a minimal rise in urinary excretion of titratable acid (from 23.0 to 35.0 μEq. per 1.73 M.^{2} per minute), and there was only a slight increase in the urinary phosphorus excretion (from 11.0 to 22.0 μEq. 1.73 M.^{2} per minute). In G. F. there was a decrease in ammonium excretion at the time of the increase in titratable acid excretion during the intravenous administration of phosphate. Table IV lists urinary ammonium excretions following an acid load at different ages derived from studies in the literature and data obtained in the present study.

DISCUSSION

The ingestion of an acid salt results in an increased urinary excretion of ammonium in premature infants, however, the extent of this response may be influenced by such factors as age (maturity), body weight, dosage and duration of administration of the acid load, degree of acidosis induced, urinary pH, possible hormonal response, and others.

1. **Age and body weight.** The urinary excretion of ammonium in the younger and smaller premature infants in this study is less than in the older and larger premature infants when compared both per unit of body weight and per unit of body surface, with one exception. These smaller premature infants showed urinary ammonium excretions comparable to those premature infants studied by Gordon and associates.[10] However, the 3 larger premature infants excreted, on an average, a greater amount of ammonium when compared per unit of body weight and body surface than the premature infants studied by Gordon[10] as well as those newborn infants studied by McCance and Hatemi,[19] but were comparable to the 2- to 4-month-old, full-term infants studied by Gordon,[10] and to the adults studied by Ryberg[20] and to the older child studied by Gamble, Blackfan, and Hamilton[21] when

Table II. Larger premature infants during calcium chloride administration

	Day	Serum			$CaCl_2$ intake (mEq.)	Vol. (c.c.)
		CO_2 (mEq./L.)	Na (mEq./L.)	Cl (mEq./L.)		
B. A. R., age 34 days, weight 2.64 kilograms, surface area 0.1966 M.²						
Base line	U_1	–	–	–	–	170
	U_2	24.8	–	112	–	228
Total		24.8	–	112	–	398
Average		24.8	–	112	–	199
$CaCl_2$ loading period	U_{13}	–	–	–	18.5	148
	U_{14}	–	–	–	18.5	280
	U_{15}	–	–	–	18.5	219
	U_{16}	–	–	–	18.5	143
Total		–	–	–	74.0	790
Average		–	–	–	18.5	198
B. U. R., aged 49 days, weight 2.5 kilograms, surface area 0.190 M.²						
Base line	U_1	–	–	–	–	211
	U_2	–	–	–	–	191
	U_3	–	–	–	–	151
	U_4	–	–	–	–	307
Total		–	–	–	–	860
Average		–	–	–	–	215
$CaCl_2$ loading period	U_5	–	–	–	17.5	255
	U_6	–	–	–	17.5	307
	U_7	–	–	–	17.5	213
	U_8	–	–	–	17.5	190
	U_{10}	–	–	–	17.5	227
	U_{11}	–	–	–	17.5	215
	U_{12}	–	–	–	17.5	233
	U_{13}	–	–	–	17.5	237
	U_{14}	–	–	–	17.5	303
	U_{15}	–	–	–	17.5	215
	U_{16}	–	–	–	17.5	212
Total		–	–	–	192.5	2607
Average		–	–	–	17.5	237
D. O. Y., aged 43 days, weight 2.36 kilograms, surface area 0.1826 M.²						
Base line	U_1	–	–	–	–	175
	U_3	–	–	–	–	196
	U_4	–	–	–	–	220
Total						591
Average		–	–	–	–	164
$CaCl_2$ loading period	U_6	–	–	–	16.8	335
	U_7	–	–	–	16.8	310
	U_8	–	–	–	16.8	195
	U_9	–	–	–	16.8	153
	U_{14}	–	–	–	16.8	202
	U_{15}	–	–	–	16.8	194
	U_{16}	–	–	–	16.8	190
	U_{17}	–	–	–	16.8	186
	U_{18}	–	–	–	16.8	210
Total					151.2	1975
Average		–	–	–	16.8	219

24-hour urine							
pH	Cl (mEq.)	NH_3 (mEq.)	Na (mEq.)	K (mEq.)	T. A. (mEq.)	O. A. (mEq.)	PO_4 (mg.)
5.7	–	2.7	4.8	4.5	1.32	–	1.38
5.6	6.0	2.8	3.6	6.0	2.18	–	1.86
11.3	6.0	5.5	8.4	10.5	3.50	–	3.24
5.7	6.0	2.8	4.2	5.3	1.75	–	1.62
5.3	10.2	8.4	3.6	3.5	2.96	–	2.06
5.2	20.7	11.5	12.1	5.6	3.50	–	2.13
5.2	18.9	10.3	10.8	7.0	3.42	–	2.03
5.2	10.5	5.4	5.7	4.2	2.17	–	1.33
20.9	60.3	35.6	32.2	20.3	12.05	–	7.55
5.2	15.1	8.9	8.1	5.1	3.01	–	1.88
5.44	3.5	3.5	1.5	7.8	4.9	–	–
5.60	3.8	3.4	3.2	5.2	4.0	–	–
6.07	4.5	2.4	4.0	3.0	2.0	–	–
5.75	9.0	4.3	6.8	6.0	4.6	–	–
22.86	20.8	13.6	15.5	22.0	15.5	–	–
5.72	5.2	3.4	3.9	5.5	3.9	–	–
5.74	14.7	6.6	8.1	6.6	3.1	–	–
5.35	21.6	9.5	12.0	7.9	4.3	–	–
5.26	21.3	10.8	6.8	3.3	3.7	–	–
5.47	18.3	14.4	3.8	3.2	3.8	–	–
5.53	19.1	15.0	5.4	3.5	3.6	–	–
5.64	16.1	14.6	4.0	4.1	4.1	–	–
5.61	16.8	12.1	5.3	6.0	4.0	–	–
5.48	18.0	12.1	6.4	5.0	3.6	–	–
5.57	25.2	13.1	11.9	6.8	3.5	–	–
5.43	13.4	9.9	5.1	6.4	3.2	–	–
5.42	15.3	11.0	4.5	7.3	3.7	–	–
60.50	199.8	129.1	73.3	60.1	40.6	–	–
5.50	18.2	11.7	6.7	5.5	3.7	–	–
5.05	4.6	3.4	3.3	4.3	3.9	–	–
4.92	5.2	4.7	3.6	3.9	4.0	–	–
6.75	–	6.6	3.4	5.0	2.7	–	–
16.72	9.8	14.7	10.3	13.2	10.6	–	–
5.25	4.9	4.9	3.4	4.4	3.5	–	–
6.63	17.7	10.7	11.8	6.4	2.0	–	–
6.21	19.7	9.9	12.7	6.5	3.0	–	–
6.39	14.2	8.6	8.1	4.1	2.2	–	–
5.79	12.1	7.2	6.3	4.2	2.3	–	–
5.57	12.1	13.2	3.0	5.1	5.0	–	–
5.35	15.0	11.1	4.3	4.5	3.6	–	–
5.52	9.7	9.2	1.4	4.7	3.8	–	–
5.56	14.1	11.9	3.2	6.0	3.7	–	–
6.25	12.0	11.3	2.9	6.6	2.8	–	–
53.27	126.7	93.1	53.7	48.1	28.4	–	–
5.91	14.1	10.3	6.0	5.3	3.2	–	–

Table III. The intravenous administration of isotonic neutral phosphate

Subject	*Day of study*	*Time (min.)*	*Plasma*			*Urinary excretion (mEq./1.73 M.²/min.)*		
			CO_2 (mEq./L.)	*Ca (mg. %)*	*P (mg. %)*	*T. A.*	*NH_4*	*PO_4*
G. F., aged 46 days, weight 2.0 kilograms, surface area 0.158 M.²	U_1	75	8.5	10.4	6.4	.029	.058	.019
			Intravenous phosphate					
	U_2	30	11.5	7.0	35.7	.117	.054	.156
	U_3	60	–	–	–	.200	.041	.330
	U_4	60	–	5.7	14.5	.105	.039	.165
	U_5	60	–	–	–	.067	.032	.099
	U_{21}	1,230	–	–	–	.025	.056	.041
	U_{22}	1,080	–	–	–	.021	.046	.030
G. M., aged 18 days, weight 1.4 kilograms, surface area 0.129 M.²	U_1	60	18.5	–	8.8	.023	.027	.011
			Intravenous phosphate					
	U_2	35	15.3	–	10.8	.015	.103	.009
	U_3	60	17.7	–	–	.035	.023	.022
	U_4	60	15.8	–	8.3	.012	.012	.009
	U_{10}	1,280	–	–	–	.021	.026	.017
	U_{11}	1,440	–	–	–	.028	.023	.025

compared per unit of body weight. In the premature infants reported by Gordon[10] the urinary ammonium/chloride ratios calculated during acid loading show values of 29 per cent, 30 per cent, and 31 per cent while the smaller premature infants in the present study show values of 29 per cent, 62 per cent, 42 per cent, and 32 per cent. The ratios in the larger premature infants in the present study are comparable to those of the newborn infants studied by Gordon and associates. Their infants, 2 to 4 months of age had ratios of 43 per cent, 75 per cent, and 47 per cent, while the values in the larger premature infants in this study are 59 per cent, 64 per cent, and 73 per cent. It is generally assumed that a ratio of 75 per cent is a good response in the adult.[30] It is apparent from these data that the larger premature infants are not deficient in hydrogen-sodium exchange when calculations are based either on ammonium excretion per unit of body weight, or the ammonium/chloride ratio.

2. **Duration of acid load administration.** A plateau effect in the maximum urinary ammonium excretion in man has previously been described by Gamble and colleagues[21] and confirmed by Ryberg[20] as occurring about the fourth day of acid loading. In 4 of 7 premature infants in this study, maximum ammonium excretions occurred after the fourth day of calcium chloride ingestion. The low values found by McCance and Hatemi[19] may be primarily due to the short period of acid administration (8 hours). Nevertheless, when compared on a weight basis, these authors note that the newborn infant does as well as the adult even in these short periods of acid loading.

3. **Degree of acidosis.** Another possible modifying factor in urinary excretion of ammonium is the degree of acidosis induced with the acid load. Fig. 1 shows an inverse relationship between the total urinary ammonium excretion and plasma bicarbonate concentrations ($P < 0.01$). The urinary pH change in these premature infants was not maximally acid and may well be a factor in the lowered ammonium excretion in the smaller premature infants, since the diffusion of ammonia is dependent on the acidity of the tubular fluid. The failure to achieve maximum urinary acidity in this study, on the other hand, is most probably related to the diminished availability of buffer salts such as sodium phosphate at the distal portion of the nephron.

4. **Hormonal effects.** Adrenalectomized rats fail to show an adequate increase of ammonia excretion in response to an acid load,[22] and this deficiency can be corrected

with either desoxycorticosterone or adrenal cortical extract. There is suggestive evidence in a preliminary and incomplete study that a similar effect may occur in premature infants.

Hydrogen ion excretion in exchange for sodium in the presence of acidosis is also accomplished by the acidification of urinary buffers, the chief one of which is phosphate. The total available urinary buffer salts in young newborn infants have been reported to be lower than that observed in the adult.[23-25] McCrory and collaborators[26] found urinary buffer salts excreted at a higher rate in newborn infants fed cow's milk as compared to fasting adults on the basis of surface area. The latter authors also showed an increased phosphate clearance following the administration of phosphate in normal premature infants. In the present study, both premature infants excreted an amount of titratable acid equal to the adult during the calcium chloride loading period, prior to the phosphate administration, when compared per unit of body surface (Table III).

Latner and Burnard,[27] in studying infants with renal acidosis, demonstrated an increase of titratable acid excretion following phosphate loading. McCance and associates[19] also showed a rise in titratable acid excretion in young newborn infants following neutral phosphate administration. The data presented in this report on 2 premature infants made acidotic with calcium chloride and following the intravenous administration of neutral phosphate show a rise in titratable acid excretion approaching that of the adult on a surface area basis in 1 of the 2 infants. To obtain these results, it was necessary to elevate the plasma phosphate concentration in the infant to approximately twice that observed in the adult with phosphate loading, or 6 times that of the normal infant (Table V). In the second infant, given a phosphate load sufficient to elevate the plasma phosphate only 20 per cent, there was a considerably smaller increase in titratable acid excretion than that of the first infant. This difference in response in the 2 infants indicates the importance of the filtered phosphate load in influencing the titratable acid excretion.

Table IV. Average daily urinary ammonium excretion

Authors	$CaCl_2$ (days)	Age (days)	Weight (kilograms)	Surface area ($M.^2$)	Subject	Urinary ammonium: Kilogram of body weight per day	Urinary ammonium: $M.^2$ per day
Smaller premature infants of present study	9	27	1.43	0.141	B. B.	1.84	18.7
	9	28	1.40	0.159	B. W.	4.77	42.2
	9	26	1.64	0.157	G. F.	2.23	23.2
	9	27	1.70	0.159	G. M.	2.10	21.5
Larger premature infants of present study	14	43	2.64	0.197	B. A. R.	3.37	45.5
	11	49	2.50	0.190	B. U. R.	4.68	62.8
	12	43	2.36	0.183	D. O. Y.	4.36	56.4
Gordon et al.,[10] premature infants	3	15	2.83	0.199	A. H.	2.30	32.5
	3	11	2.31	0.172	M. R.	2.56	34.5
	3	45	2.10	0.158	S. L.	2.38	31.6
Gordon et al.,[10] newborn infants	3	122	6.03	0.350	D. H.	2.87	49.5
	3	100	4.70	0.290	D. F.	4.13	67.1
	3	51	3.99	0.251	D. S.	3.21	51.1
McCance et al.,[19] full-term infants	8 hours	7	–	–	–	–	12.7
Gamble et al.[21]	6	8 years	27.0	1.00(?)	I	2.78	75.0
Ryberg[20]	16	Adult	53.0	1.73(?)	I	3.89	119.0
	7	Adult	53.0	1.73(?)	I	3.61	112.0

Table V. Comparison of intravenous phosphate administration in an adult and a premature infant

	Plasma (mm./L.)		*Urinary excretion (mEq./1.73 M.²/min.)*		
	PO_4	HCO_3	PO_4	*T. A.*	NH_4
Premature infant	2.06	11.5	.019	.029	.058
			Intravenous phosphate		
	11.52	–	.330	.200	.041
Adult[8]	1.10	14.0	.016	.027	.060
			Intravenous phosphate		
	6.52	–	.346	.333	.056

Elkington and co-workers[28] have used what they term "clearance index of hydrogen ions" ($UV_{H^+} = UV_{TA} + UV_{NH_4^+} - UV_{HCO_3^-}$) as a quantitative assessment of the renal excretion of hydrogen ions. After the third day of an acid load, the values for the normal adult varied between 1.4 and 3.4. Applying this formula to the data presented by Gordon,[10] the premature infants had an average of 1.89 and the full-term infants 2.58. The smaller premature infants in our study, on the third day of acid loading, had values of 0.62, 1.84, and 0.98, which suggests that the hydrogen-sodium ion exchange in some smaller premature infants may be relatively inefficient in compensating for acidosis. The data showing the larger titratable acid excretion in the premature infant, made possible by the phosphate loading, would indicate that the clearance index of hydrogen ions is not a maximal value, for with phosphate loading, a higher index might be anticipated.

A converse of hydrogen ion excretion during acidosis is the urinary sodium excretion, the major cation for which hydrogen ions are exchanged in the process of base conservation. The smaller premature infants in this study during severe acidosis excreted sodium at an average of 1.5 mEq. per kilogram per day above baseline values, while the larger premature infants averaged 1.2 mEq. per kilogram per day above baseline values. The larger sodium loss in premature infants, as compared to older infants, is more apparent in the data presented by Gordon and associates.[10]

CONCLUSIONS

It is apparent that the smaller premature infant, 1 month of age and weighing approximately 1.5 kilograms, is not as efficient as the larger premature infant, older child, or adult in the production of hydrogen ions in binding ammonia, either on a weight or surface area basis, under the stress of acidosis induced by calcium chloride. The larger and somewhat older premature infants respond to prolonged acid loading in a manner comparable to that of adults, when the values are calculated on a weight basis,[20] and to breast-fed infants, 3 to 5 months of age, when calculated on a surface area basis, as reported by Foman, Harris, and Jensen.[29] The quantity of ammonium excreted per unit of body weight by the larger premature infants in this study was greater than that excreted by the premature infants studied by Gordon although the infants of the 2 groups were similar in weight. On the basis of surface area the premature infants in this study excreted ammonium in quantities comparable to those excreted by the full-term infants in the study of Gordon. It may be reasonable to point out that the premature infants described by Gordon did not develop severe acidosis, as indicated by blood CO_2 values of 20, 21, and 23 mEq. per liter, nor were they maintained on prolonged acid loading. Thus many variables may influence the amount of ammonium excretion in premature infants: age, weight, and the degree of acidosis.

These studies suggest that the phosphate load is a limiting factor in the excretion of

hydrogen ion by the kidney in the premature infant. When this limitation is removed by the administration of a phosphate load, the potential for an increased titratable acid excretion is greater and approaches that observed in the adult per unit of body surface.[20]

In a study of premature infants weighing over 2 kilograms and comparable in weight and age to the larger premature infants in this study, Tudvad, McNamara, Barnett[9] present data showing tubular bicarbonate reabsorption comparable to the adult per unit of glomerular filtrate. The effectiveness of smaller premature infants in this aspect of hydrogen ion exchange needs further study.

SUMMARY

1. Small premature infants, weighing approximately 1.5 kilograms and about 4 weeks of age, are relatively inefficient in hydrogen-sodium ion exchange with regard to ammonium excretion in the presence of induced acidosis. Larger premature infants, weighing approximately 2.5 kilograms and about 6 weeks of age, are more mature in this capacity and are comparable to older children and adults when compared per unit of body weight. On a surface area basis, the larger premature infants are also comparable to full-term infants 2 to 4 months old in excretion of ammonium.

2. The filtered phosphate load is a limiting factor in the amount of titratable acid excreted in the small premature infant during induced acidosis. In the larger premature infant, the urinary titratable acid excretion is comparable to that of the adult on a surface area basis both before and after phosphate loading.

3. The smaller premature infant may be handicapped in his defense against acidosis through a deficiency in renal function.

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Excretion of D- *and* L-*methionine in children with generalized aminoaciduria*

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CLEARANCE OF D- AND L-METHIONINE IN CHILDREN WITH GENERALIZED AMINOACIDURIA

THE aminoaciduria associated with glycosuria and phosphaturia in the Fanconi syndrome or in patients with mental retardation and congenital cataracts as described by Lowe, Terrey, and MacLachlan[1] is generally agreed to be the result of insufficient reabsorption of amino acids by the renal tubule. The nature of the defect of the renal tubule, however, is unknown at the present time.

The reabsorption by the kidney of the naturally occurring L-form of the individual amino acid is thought to be a process of active transport which involves, perhaps, a mechanism of the membrane carrier type.[2-4] The reabsorption of the D-isomers appears to be quantitatively if not qualitatively different.[4, 5] Because of the apparent difference in the handling of the 2 isomers by the normal renal tubule, it was considered to be of interest to compare the reabsorption of the D- versus the L-form of an amino acid in patients with generalized aminoaciduria as a means of defining further the nature of the renal tubular abnormality in these syndromes.

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Observations on the patients in this study were performed at Stanford University Hospital from 1954 through 1957.

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CASE MATERIAL

Clearance studies were performed on 8 children. Four had cystinosis and the well-recognized renal tubular reabsorptive defects of the Fanconi syndrome: phosphaturia, glycosuria, hyperchloremic acidosis, and a generalized aminoaciduria; 2 were considered to be variants of the Lowe syndrome, with retardation, congenital cataracts, and generalized aminoaciduria. One child with healed acute glomerular nephritis, and 1 with orthostatic albuminuria served as controls. Brief clinical summaries of these children follow.

Fanconi syndrome with cystinosis. D. E. was a male mulatto born May 8, 1951, weighing 2,727 grams. The pregnancy was full term and uneventful, as was the delivery. This infant sat at 6 months, walked

Table I. Laboratory data in Fanconi and Lowe syndromes

		Fancon	
		D. E.	*D. W.*
Weight (kilograms)		11.7	6.0
Blood			
Bicarbonate	mM./L.	19.1	14.0
Chloride	mEq./L.	118.8	122.0
Sodium	mEq./L.	139.0	
Potassium	mEq./L.	3.9	
Urea nitrogen	mg./100 ml.	64.0	21.0
Creatinine	mg./100 ml.	1.3	0.6
Cholesterol	mg./100 ml.	305.0	321.0
Total protein	Gm./100 ml.	7.3	
albumin	Gm./100 ml.	4.6	
globulin	Gm./100 ml.	2.7	
Calcium	mg./100 ml.	11.4	10.1
Phosphorus	mg./100 ml.	3.5	2.2
Alkaline phosphatase	(Bodansky Units)	8.7	23.1
Amino acid nitrogen	mg./100 ml.	5.4	5.9
X-ray of bones		Rickets Delay in maturation	Rickets
Urine			
Specific gravity		1.009 to 1.012	
Protein	Gm./24 hr.	2.0	0.175
Sugar	Gm./24 hr.	0 to 0.600	1.8 to 6.0
Phosphorus	mg./24 hr.		444.0
Urea nitrogen	Gm./24 hr.	4.9	
Amino acid nitrogen	mg./24 hr.	163 to 238	278

at 8 months, and spoke single words shortly thereafter. Urinary frequency was noticed at 1 year. This persisted and seemed to increase simultaneously with failure to gain weight for about 4 months prior to the first hospitalization. A paternal nephew was thought to have the same symptoms. He was hospitalized at Stanford University Hospital on Feb. 1, 1954, at the age of 2 years, 9 months, with the tentative diagnosis of diabetes insipidus. Physical examination revealed a small, underweight child with a liver which was palpable 2 cm. below the costal margin. Blood pressure was 90/68. The laboratory studies on admission are summarized in Table I. There was no concentration of urine after 10 hours of fluid restriction. Specific gravity of the urine remained 1.009 and the packed red cell volume rose from 38 to 42. No response was elicited with vasopressin. Urea clearance was 33 ml. per minute per 1.73 M^2, and the excretion of phenolsulfonphthalein (PSP) was only 5 per cent in 2 hours. A conjunctival biopsy established the diagnosis of cystinosis. Treatment with Shohl's solution,* added potassium, and large amounts of vitamin D produced some improvement in the osteomalacia. Subjectively, he was better although there was little gain in height or weight. During the last year of life there was marked acceleration in renal failure and he died on Mar. 30, 1961, at the age of 9 years, 11½ months.

D. W. was a Caucasian female born Oct. 30, 1954. This infant was seen initially at Stanford University Hospital on April 2, 1955, at the age of 5 months, with the history of seizures of 1 month's duration. She was the product of an uneventful pregnancy and delivery and was entirely well until 4 months of age, at which time she developed convulsions and seemed to "stop playing with her hands." Family history was noncontributory. Physical examination was negative except that the head measured only 37 cm. in

*Shohl's solution: sodium citrate 98 Gm., citric acid 140 Gm., water q.s. 1,000 ml.

ndrome		Lowe syndrome	
C. D.	L. P.	R. G.	R. J.
18	16	15	13
11.3	15.2	22.5	20.5
110.0	106.0	103.2	103.6
137.0	144.0	143.0	142.5
3.6	3.5	4.6	4.0
	149.0	29.0	
5.5	4.1	0.6	
264.0	443.0		
	7.4	7.9	7.3
	4.4	4.6	4.1
	3.0	3.3	3.2
7.5	6.1	10.3	9.8
5.3	5.7	3.8	3.2
14.9	14.7	9.2	17.1
Rickets Delay in maturation	No rickets Delay in maturation	No rickets Delay in maturation	Normal
1.010	1.014	1.028	1.010 to 1.020
1.46	3+	0.684	2+ to 3 +
0.206	0	0	0
137.5	310	469.8	
	7.4	8.41	8.51
62 to 38	64	143	258 to 554

circumference. Electroencaphalogram showed the presence of bilateral, synchronous, high voltage multiple spike and wave complexes. Spinal tap was negative. Urinalysis revealed glycosuria and proteinuria. At the time of rehospitalization, 4 months later (Aug. 10, 1955), gross retardation was evident. More complete studies (summarized in Table I) substantiated the diagnosis of the Fanconi syndrome. She was treated with M/6 lactate and barbiturates. In spite of this therapy, she continued to experience 3 to 4 generalized seizures weekly. On Dec. 13, 1955, she was rehospitalized for renal studies, after which she was sent to the Sonoma State Hospital where she died shortly thereafter. Postmortem examination revealed cystinosis.

C. D., hospitalized at Stanford University Hospital on Jan. 16 to 18, 1957, for renal studies, was a Caucasian female born on April 12, 1948, weighing 4,090 grams. Apparently she was well until 1 year of age, at which time polyuria was noticed. At 2 years of age she developed pneumonia and became severely dehydrated. The diagnosis of diabetes insipidus was made and for the subsequent 2½ years she received vasopressin which seemingly diminished the output of urine. For the next 3½ years she remained without medication, with no alteration in symptoms except a questionable increase in polyuria. She then started school and became aware of photophobia. In March, 1956, a 2-year-old sibling died in renal failure and cystinosis was discovered at autopsy; 4 other siblings were all normal. C. D. was then hospitalized at the University of California, where the diagnosis of the Fanconi syndrome with cystinosis was made. She was quite short; at the age of 9 years she had a bone age of 5 years, 3 months. Pertinent laboratory data are summarized in Table I. She received supportive therapy during the last 18 months of life and died in renal failure on Sept. 4, 1957, at the age of 9 years, 5 months.

L. P. was a Caucasian female who was born Jan. 4, 1949 (weight 3,070 grams) after

Table II. Renal excretion of L-methionine at endogenous and elevated serum L- and D-methionine concentrations*

Period	C_{IN} (ml./min.)	*Serum* L-*methionine* (mg./ml.)	L-*methionine (mg./min.)*			C_{Lm} (ml./min.)	D-*methionine*			C_{Dm} (ml./min.)
			Filtered	*Excreted*	*% reabsorbed*		*Filtered*	*Excreted*	*% reabsorbed*	
1		.007								
	Priming dose of 10 ml. of 10% inulin followed by sustaining infusion of 10 ml. of 10% inulin in 200 ml. of saline dripping at 1 ml./min.									
2	8.58	.0055	.04719	.0035	92.58	0.64				
3	6.79	.0055	.03735	.0030	91.97	0.55				
4	6.94	.0055	.03817	.0035	90.84	0.64				
	Priming dose of D- and L-methionine, 6 ml. of 3% solution followed by sustaining solution of inulin and saline and 1½% D- and L-methionine dripping at 1 ml./min.									
5	7.28	.100	.7280	.2375	67.38	2.38	1.354	1.063	21.49	5.72
6	7.16	.115	.8234	.3425	59.40	2.98	1.575	1.708		7.76
7	6.64	.132	.8765	.4050	53.79	3.07	1.726	1.72	0.35	6.62

*D. W., white female, with Fanconi syndrome with cystinosis; weight 6.010 kilograms.

a normal full-term pregnancy and normal delivery. The early childhood was uneventful except for frequent upper respiratory infections. Her parents were not concerned about her small stature until the age of 7 years, when a sibling 2 years her junior surpassed her in height. In February, 1956, she was seen by her local physician who made the diagnosis of chronic glomerular nephritis on the basis of dwarfism and the following results of chemical studies of the blood: hemoglobin 8.5 Gm. per 100 ml., urea nitrogen 31 mg. per 100 ml., creatinine 1.8 mg. per cent, calcium 10 mg. per cent, phosphorus 4.3 mg. per cent, chloride 111 mEq. per liter, potassium 3.4 mEq. An intravenous pyelogram showed small kidneys with poor concentrating ability. The bone age was 3 years, 9 months at a chronologic age of 7 years. In November, 1956, she was referred to Stanford University Hospital for evaluation of the kidney function. Laboratory findings are summarized in Table I. Cystinosis was confirmed by corneal biopsy. Despite supportive therapy, she developed progressive renal insufficiency and died Jan. 15, 1958, at the age of 9 years.

Lowe syndrome. R. G., sent to Stanford

Table III. Excretion of amino acids in children with generalized aminoaciduria (mg./24 hr.)

Amino acid	*Age control 3 years old*	*Fanconi syndrome*		*Lowe syndrome*
		D. E. 3 years	*D. W. 10 months*	*R. J. 2½ years*
Glycine	41.0	114		630
Glutamine and glutamic acid	11.0			394
Histidine	49.0	27		280
Threonine	1.6	96	112	175
Lysine	2.0	58	128	367
Serine	5.0			290
Tyrosine	5.0			131
Proline	10.0			47
Valine	4.0		69.6	13
Alanine	1.5			308
Cysteine/cystine	10.0	97.2	77	175
Phenylalanine	3.0	15		44
Tryptophan	6.0	24	19.3	41
Arginine	3.0	15.6	39.3	131
Leucine	1.5	33	40.5	27
Methionine	0.2	3.3	11.2	17
Isoleucine	1.7	12	18.5	15

Table IV. Excretion of urinary amino acid nitrogen in D. E. (Fanconi syndrome with cystinosis)

Date	Urine volume (ml./24 hr.)	Amino acid nitrogen (mg./24 hr.)	Treatment per 24 hours
Feb. 12, 1954		163.0	Shohl's solution 30 ml. and vitamin D 5,000 units
May 25, 1954		238.8	
Oct. 6, 1955	2,135	56.0	Shohl's solution 60 ml., potassium 135 mEq., vitamin D 50,000 units
Oct. 7, 1955	1,970	70.0	
Nov. 9, 1955	1,870*	27.0	
Dec. 1, 1955	1,840*	67.0	
Dec. 29, 1955	2,000*	44.0	No vitamin D from Dec. 28, 1955 to Jan. 11, 1956
Jan. 15, 1956	3,100	130.00	

*One specimen lost during night.

University Hospital at 1½ years of age after experiencing 3 unsuccessful cataract extractions, was a Mexican boy who was born on Nov. 10, 1950. Pregnancy was full term and normal; delivery was uneventful. Bilateral cataracts and nystagmus were noticed in the first weeks of life. One uncle had died in infancy with congenital cataracts. The infant was extremely retarded; he rolled over at 1 year, sat without support at 3 years, stood and walked only with support at 5½ years. At 3 years he experienced generalized convulsions with febrile episodes, which were controlled with phenobarbital. At 5 years, 10 months, the time of the renal studies, he spoke only single words and was not toilet trained. He was hospitalized repeatedly for eye operations; finally one eye had to be enucleated because of uncontrollable secondary glaucoma and staphyloma. Pertinent laboratory data are found in Table I.

R. J., initially seen at Stanford University Hospital at the age of 2 years, 3 months with the history of "crossed eyes since 5 months of age and a white spot in eye noticed at 1 year of age," was a Negro boy born May 18, 1951 (weight 3,090 grams). The mother was pre-eclamptic during pregnancy which, however, was full term. The delivery was normal. The father's first cousin had cataracts in infancy. The infant was retarded; he sat at 11 months, walked at 23 months, and at 28 months spoke only a few words. He was hospitalized repeatedly for removal of the bilateral cataracts and treatment of secondary glaucoma (Table I).

Controls. C. A. was a Caucasian male born Jan. 4, 1947, who had acute glomerular nephritis on Feb. 14, 1955, but recovered promptly and by May 17, 1955, had normal Addis counts. The amino acid studies were performed on Sept. 13, 1955.

Y. M. was a Japanese female born Jan. 4, 1941. She had orthostatic albuminuria which was found incidentally and diagnosed in July, 1954. The renal studies were performed in Oct. 1955.

METHOD

All clearance procedures were performed in the resting-fasting state. The children were encouraged to drink 200 to 500 ml. water before the beginning of the study. Urines were collected by catheter in periods of 10 to 15 minutes. The bladders were washed with 8 to 12 ml. distilled water followed by 5 to 15 ml. air. Blood samples were drawn at the beginning and close of each group of 3 urine samples. The midpoint of each period was computed. After control periods, a priming dose of 10 per cent inulin was given intravenously and a solution of inulin in saline, which was designed to maintain a constant blood level of inulin, was allowed to flow at 1 ml. per minute by gravity drip. Approximately 40 minutes were allowed for equilibration of inulin. After 3 urine samples were obtained for determina-

Table V. Clearance of inulin and urine flow in children with generalized aminoaciduria

Subject	*Surface area* (M^2)	*Clearance of inulin (ml./min.)**		*Urine flow* (ml./min.)*	
		Control	*Load*	*Control*	*Load*
Fanconi syndrome					
D. E.	0.54	13.8 ± 1.17	12.8 ± 2.06	2.8 ± 0.21	2.8 ± 0.71
D. W.†	0.25	7.4 ± 1.01	7.0 ± 0.54	Constant at approximately 0.8	
C. D.	0.69	3.8 ± 0.50	4.7 ± 1.00	1.0 ± 0.07	1.2 ± 0.39
L. P.	0.65	2.6 ± 0.12	3.0 ± 0.12	1.4 ± 0	1.6 ± 0.10
Lowe syndrome					
R. G.	0.60	44.8 ± 1.89	38.1 ± 14.20	0.5 ± 0	0.33 ± 0.09
R. J.	0.54	33.5 ± 3.78	28.5 ± 5.77	1.1 ± 0.27	0.63 ± 0.12
Normal					
C. A.	1.05	69.4 ± 1.86	67.7 ± 4.82	1.9 ± 0.26	1.6 ± 0.57
Y. M.	1.44	93.1 ± 8.52	69.7 ± 14.8	3.5 ± 0.25	5.1 ± 1.9

*All values are means with standard error of 3 collection periods.
†Added water not accurately recorded.

tion of clearance of inulin and endogenous methionine, a priming injection of 3 per cent DL-methionine (20 ml. per square meter) was given, followed by a mixture of inulin and 1½ per cent DL-methionine adjusted for maintenance of constant blood concentrations. This infusion was also allowed to flow at 1 ml. per minute by gravity drip. A period of 20 minutes was allowed for equilibration of the amino acid. This amount of methionine and the period of equilibration were chosen arbitrarily, on the basis of previous experience which indicated the results would be satisfactory for these studies.[6] An example of such a procedure is tabulated in Table II. Glomerular filtration rate was measured by means of the clearance of inulin.[7] The values were uncorrected for surface area. L-methionine was measured by microbiologic assay with the use of *Leuconostoc mesenteroides*. The value for D-methionine was obtained by subtracting the value for L-methionine from that of DL-methionine which was obtained with the use of *Lactobacillus fermenti*.

RESULTS

1. Clinical observations in patients with generalized aminoaciduria. D. E., C. D. and L. P. illustrate the characteristic signs, symptoms, and clinical course of the Fanconi syndrome with cystinosis (Table I). Each was dwarfed and had hyperchloremic acidosis, hypophosphatemia, hypokalemia, glycosuria, aminoaciduria, and polyuria; D. E. and C. D. had rickets, L. P. did not. The presence of cystine storage was established in each by observation of cystine crystals in the cornea by slit lamp or biopsy and by postmortem examination. The generalized nature of the aminoaciduria is illustrated in Table III.

C. D. and L. P. were not diagnosed correctly and hence were not treated very vigorously until they were 8 and 7 years old, respectively, and already had evidence of severe renal impairment (creatinine concentrations of 5.5 and 4.1 mg. per 100 ml. in the serum). With this degree of glomerular function, it is not uncommon to find that the phosphorus concentration in the serum is increased to normal values and the rickets are healed. It is of interest that C. D. had active rickets and L. P. did not. Each child received alkali therapy and vitamin D during the last 12 to 18 months of life; however, only slight clinical benefit was experienced and each died in renal failure.

D. E., on the other hand, was diagnosed at the age of 2 years, 9 months, at a time when the serum creatinine was only slightly

elevated, 1.3 mg. per 100 ml. He was started on supportive therapy, which was faithfully administered as prescribed. On large doses of vitamin D, diminished excretion of amino acids occurred (Table I). The occurrence of this has been reported by Bickel[8] in the treament of children with the Fanconi syndrome and cystinosis, and by Salassa[9] who observed this change in older children with the Fanconi syndrome in whom cystinosis was not demonstrated.

Reduction in filtration rate, which has been shown to occur in some instances with massive doses of vitamin D,[10] could have accounted for the reduced excretion of amino acids. The clearance studies were done Oct. 7, 1955, during this period of large dosage. Unfortunately, none were done before or afterward. However, determinations of blood urea nitrogen before, during, and after the massive therapy were 59 mg. per 100 ml. (May 17, 1954), 47 mg. per 100 ml. (Oct. 25, 1955), and 52 mg. per 100 ml. (April 4, 1956), respectively.

In the last year of life, during which time there was rapid loss of renal function, vitamin D was discontinued because serum calcium and phosphorus were both elevated (5.6 mEq. per liter and 10.5 mg. per 100 ml.) and it was feared that metastatic calcification would occur. In spite of apparent adequate supportive therapy, D. E. did not survive much longer than the 2 patients with relatively little treatment. At the time of severe loss of filtration, the total urinary amino acid nitrogen was found to be almost within normal limits. This is well illustrated by C. D. and L. P. A similar situation has been reported by Weber[11]; however, he interpreted his finding as illustrative of a patient with cystinosis without reference to the reabsorptive defects of the Fanconi syndrome.

D. W., in addition to the tubular defects and cystinosis, was also severely mentally retarded. This is not a usual finding in this disease, so that simultaneous occurrence of these defects in this patient may be coincidental. It should be recalled, however, that a generalized aminoaciduria has been reported in association with abnormalities of the eye and mental retardation such as observed in R. G. and R. J. in this series, and in patients described by Lowe,[1] Bickel and Thursby-Pelham,[12] and Thelander.[13]

R. G. and R. J. had severe mental retardation and congenital cataracts and seemed to resemble the patients described by Lowe,[1] although they did not have hyperchloremic acidosis. The aminoaciduria was found to be as extensive as that associated with the Fanconi syndrome (Table III). It has not

Table VI. Plasma concentration, filtered load, and excretion values of L-methionine

Subject	Plasma concentration (mg./100 ml.)				Filtered load (mg./min.)				Excretion (mg./min.)			
	Fasting	Period after loading			Fasting	Period after loading			Fasting	Period after loading		
	*	4	5	6	*	4	5	6	*	4	5	6
Fanconi syndrome												
D. E.	0.52	6.67	8.35	10.60	0.063	0.745	1.260	1.275	0.003	0.068	0.110	0.140
D. W.	0.55	10.00	11.50	13.20	0.041	0.728	0.823	0.877	0.003	0.238	0.343	0.405
C. D.	0.50	3.88	4.45	5.02	0.019	0.136	0.200	0.285	0.005	0.041	0.050	0.082
L. P.	0.24	2.62	3.15	3.71	0.006	0.077	0.092	0.114	0.002	0.047	0.057	0.078
Lowe syndrome												
R. G.	0.40	3.84	4.20	4.60	0.179	0.972	1.497	2.456	0.003	0.018	0.031	0.027
R. J.	0.40	1.98	2.23	2.46	0.134	0.436	0.734	0.755	0.003	0.030	0.050	0.050
Normal												
C. A.	0.50	3.04	3.20	3.38	0.347	1.917	2.325	2.273	†	0.032	0.038	0.037
Y. M.	0.60	4.53	4.55	4.58	0.559	2.430	3.399	3.724	†	0.064	0.064	0.068

*Fasting values are means of three collection periods.
†Amount of methionine excreted too little for accurate measurement.

been possible, as yet, to observe the influence of vitamin D on the aminoaciduria which was exhibited in these children.

2. **Filtration rate and urine flow.** The clearances of inulin during 3 control periods and after loading with DL-methionine are summarized in Table V. There was approximately 3 times as much variation in the collection periods after loading than before, in R. G., R. J., C. A., and Y. M. In spite of the technical problems of accurate collections, the filtration rate did not change appreciably in any child, with the possible exception of Y. M.

The patients with the Lowe syndrome had normal glomerular function; those with the Fanconi syndrome had the function reduced to less than 40 per cent of adult normal, if corrected to 1.73 M^2.

Urine flow, which was not measured as carefully as possible, did not appear to be altered appreciably throughout the procedure in any single individual (Table V). This is important in evaluating the data, as water diuresis has been shown to increase the clearance of D forms of amino acids.[2, 4]

3. **Endogenous clearance of methionine.** It is of interest that the concentration of methionine in the sera of all the children with aminoaciduria was within normal limits, in spite of the extreme depression of filtration rate in some (Table VI). The clearance of L-methionine is of the same order in all of the patients (Table VII). Reabsorption was greater than 90 per cent in 4, but was depressed in 2 (C. D. and L. P.); the amount of methionine excreted was minimal in all 6, 0.002 to 0.005 mg. per minute (Table VI). C. D. and L. P. had the lowest filtration rates, hence the filtered load which is derived by multiplying the filtration rate by the concentration in the serum was necessarily small. The larger portions of these small amounts which were filtered and which appeared in the urine may indicate a greater tubular defect in these 2 children than in the others. The amount of methionine in the urine of the 2 normal children was below the limits of accuracy of measurement.

4. **Clearance of DL-methionine after loading.** The procedure was designed so that the concentration of L-methionine would be raised at least fivefold and maintained at a constant value throughout 3 periods of urine collection (Table VI). Relatively constant concentrations were achieved in the normal children and the clearance of L-methionine then became measurable (1.1 and 1.4 ml. per minute) and reabsorption was practically complete. The concentration of methionine increased during the collection periods in the blood of the children with aminoaciduria. This was particularly notable in those patients with reduced filtration rates and can probably be attributed to that fact. The clearance of L-methionine increased with

Table VII. Clearance of DL-methionine and per cent reabsorbed

	L-methionine				*D-methionine*	
	Fasting		*After loading*			
Subject	*Clearance (ml./min.)*	*Reabsorbed (%)*	*Clearance (ml./min.)*	*Reabsorbed (%)*	*Clearance (ml./min.)*	*Reabsorbed (%)*
Fanconi syndrome						
D. E.	0.73	94.7	1.2	90.3	7.9	37.4
D. W.	0.61	91.8	2.8	60.2	6.7	7.3
C. D.	1.03	73.0	1.2	72.0	2.5	44.0
L. P.	0.72	72.45	1.9	36.5	0.76	74.2
Lowe syndrome						
R. G.	0.83	98.1	0.6	98.3	–	–
R. J.	0.66	98.2	1.9	93.2	14.9	47.0
Normal						
C. A.	*	*	1.1	98.4	17.4	74.2
Y. M.	*	*	1.4	97.9	34.6	34.6

*Amount of methionine excreted below limits of accuracy of measurement.

Table VIII. Plasma concentration, filtered load, and excretion value of D-methionine

Subject	Plasma concentration (mg./100 ml.)			Filtered load (mg./min.)			Excretion (mg./min.)		
	4	5	6	4	5	6	4	5	6
Fanconi syndrome									
D. E.	14.4	17.3	20.0	1.609	2.611	2.400	1.095	1.370	1.620
D. W.	18.6	22.0	26.0	1.354	1.575	1.726	1.063	1.708	1.720
C. D.	7.8	8.3	8.8	0.273	0.374	0.502	0.142	0.209	0.293
L. P.	9.2	9.9	10.4	0.267	0.288	0.322	0.052	0.081	0.097
Lowe syndrome									
R. G.	–	–	–	–	–	–	–	–	–
R. J.	4.3	5.6	7.1	0.947	1.844	2.178	0.577	1.050	0.882
Normals									
C. A.	6.3	6.8	7.4	3.972	4.941	4.962	1.085	1.200	1.280
Y. M.	5.7	6.0	6.2	3.019	4.445	5.049	2.080	1.940	2.150

loading in all the subjects except R. G. (Table VII). Characteristically, the renal tubule responds to an increased filtered load of an amino acid by an increase both in the amount of amino acid reabsorbed as well as that excreted. With high loads, however, the percentage of the filtered load which is reabsorbed decreases.[3] This normal response occurred in all of our patients. It is of interest that at filtered loads which were relatively comparable to the others, D. W. and L. P. demonstrated a much greater reduction in percentage of reabsorption. This suggests that, in addition to the observed severe reduction in glomerular filtration, there was also more extensive involvement of the tubules.

As previously noted, the clearance of the D-isomer of methionine is normally considerably greater than that of the L-isomer. This was observed in all of the patients except L. P. (Tables VII and VIII). It should be recalled that the concept of "clearance" involves the assumption that all of a given substance which is filtered and is not recovered in the urine, is probably reabsorbed. If, however, the substance under study should undergo a metabolic conversion in the course of its passage through the kidney, so that recovery in the urine is therefore reduced, this phenomenon would result in a low clearance of questionable significance. Such a hypothesis might be invoked to explain the results of measurement of clearance of D-methionine in L. P.

The increased clearance for Y. M. may have resulted from the fact that the urine flow was greater than that of the other patients (Table V). There is no readily apparent suggestion, however, for the almost complete excretion of the filtered D-isomer in D. W. The possibilities are that the procedure was done when she was only 11 months old and/or the fact that extreme interstitial fibrosis was seen post mortem. Unfortunately, the urine flow was not measured accurately, but it certainly was not great and could not have been a factor in this finding.

DISCUSSION

In these studies, 4 of 6 patients with generalized aminoaciduria responded to intravenous loading with L-methionine essentially as a normal individual does. Although a small increment in excretion occurs as the filtered load is increased, the amount reabsorbed is also increased so that the percentage of reabsorption declines only very slowly. These observations were made with the aid of microbiologic techniques to determine methionine. With the use of paper chromatography, Lathem, Baker, and Bradley[14] made similar observations on patients with aminoaciduria of the Fanconi syndrome and of Wilson's disease. They em-

ployed intravenous infusion of protein hydrolysates to increase the filtered load of amino acids. Similar normal responses have been reported in Wilson's disease following the ingestion of protein foods,[15] and in the Fanconi syndrome when methionine was given orally.[16] Also using microbiologic techniques, Shreeve and colleagues[17] found an analogous response in patients with nephrosis who were given amino acids intravenously. However, 2 patients (D. W. and L. P.) failed to respond to amino-acid loading, excreting a much larger portion of the load than the other 4. This may be interpreted as evidence for the existence of a more extensive tubular defect which is possibly incurred with progressive total renal failure.

Under normal circumstances the tubular reabsorption of D-methionine is less than that of the L-form. All of these children responded in this normal fashion, except L. P. This characteristic response has been interpreted by Crampton[4] as indicating that the D-form of amino acids is reabsorbed by passive diffusion. The influence of water diuresis on increased excretion of the D-form is interpreted similarly.[2] Observations made on patients with cystinuria, however, indicate that passive diffusion plays virtually no role in tubular reabsorption of at least 3 of the 4 amino acids involved in that disease: cystine, lysine, and arginine. Dent[18] has shown that all cystine which is filtered is excreted both at fasting concentrations and after loading. In addition, there is no difference in the D- and L-form, both are totally excreted. Decreased excretion of D-isomer would be expected if passive diffusion were playing a role in reabsorption. Doolan[5] has shown that lysine and arginine, likewise, are not reabsorbed in cystinuria and postulates that the inability to reabsorb the involved amino acids may be a lack of specific chelation which is essential for absorption. He interprets his data as supporting the concept of a transport mechanism essential for absorption of the L-form, for which the D-isomer has a weaker affinity. Kinsell and colleagues[6] gave DL-methionine to patients with cirrhosis of the liver and were unable to account for the rate of disappearance of the D-form on the basis of renal excretion alone. They postulated that the D-isomer was catabolized by a specific enzyme, D-amino acid oxidase, known to be present in the liver and kidney. It is difficult to explain the extremely small amount of D-methionine found in the urine of L. P. as resulting from increased tubular reabsorption when it is recalled that the tubule of this patient responded so poorly to loading even with the natural isomer, L-methionine.

The data presented in these studies may be interpreted as supporting one or the other of at least two hypotheses: either the renal tubular defect is partial, even though involving all of the nephrons and capable of responding to loads, or only a small per cent of the nephron population is seriously defective. It was previously postulated by Lathem, Baker, and Bradley[14] that only a small per cent of the nephrons are involved. They supported this hypothesis by finding a normal maximal tubular capacity for reabsorption of glucose in their patient with the Fanconi syndrome. Either abnormality would result in the spilling of many amino acids in the urine under endogenous conditions. However, the observation in these children with generalized aminoaciduria that, with progressive loss of kidney function, an increasing percentage of the filtered load is excreted, leads to the interpretation that the former hypothesis is more tenable. A more specific definition of the abnormality which produces generalized aminoaciduria in the Fanconi and Lowe syndromes is not possible unless maximal tubular reabsorptive capacity for amino acids could be demonstrated in man.

CONCLUSION

Clinical observations as well as the measurement of the clearance of D- and L-methionine were made on 4 children with the Fanconi syndrome and 2 with the Lowe syndrome.

The results of a study of simultaneous clearances of inulin and endogenous methionine indicate that there is a minimal in-

crease on the clearance of methionine in the children with aminoaciduria, in contrast to normal ones. Four of the 6 patients, however, responded normally to intravenous loading with DL-methionine, i.e., as the filtered load increased, the amounts of L-methionine excreted and reabsorbed both increased, while the per cent reabsorbed remained only slightly reduced. The failure of 2 patients to exhibit the expected tubular response to loading of this degree is interpreted as evidence for the existence of extensive tubular damage.

More D- than L-methionine was excreted by 5 of these children. This is also the usual finding in normal individuals and in patients with generalized aminoaciduria. In 1 child with the Fanconi syndrome only a small amount of the filtered D-isomer was recovered in the urine. There is no acceptable explanation for this finding.

The data are interpreted as supporting the hypothesis that the tubular defect which is responsible for generalized aminoaciduria in the Fanconi and Lowe syndromes is quite minimal and probably involves the entire nephron population; as renal damage advances in the course of the disease, the ability of the tubule to respond to loading is decreased.

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Variability in the clinical manifestations of galactosemia

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GALACTOSEMIA is a hereditary disorder of carbohydrate metabolism characterized by vomiting, diarrhea, jaundice, poor weight gain, and malnutrition during early infancy. Mental retardation, cataracts, and cirrhosis of the liver may also be seen. Clinically, the diagnosis is usually established on the basis of an abnormal galactose tolerance curve and the presence of galactose in the urine. The frequent occurrence of this condition in siblings together with its reported equal distribution in both sexes suggests that galactosemia is probably transmitted by a single autosomal recessive gene.

In 1956, Schwarz and his co-workers[1] showed that galactose-1-phosphate accumulates in the erythrocytes of affected children who receive galactose or milk. In the same year, Kalckar, Anderson and Isselbacher[2] demonstrated a deficiency of *galactose-1-phosphate uridyl transferase* in the red blood cells of these patients. This indicated that the site of the metabolic block in the disease was at the step of:

$$\text{galactose-1-PO}_4 + \text{UDP glucose} \rightleftharpoons \text{UDP galactose} + \text{glucose-1-PO}_4$$

Recently, we have had the unusual opportunity of making direct observations on 45 affected individuals from 27 families and the present paper will report on the clinical manifestations seen.

MATERIALS AND METHODS

The distribution of the cases in the families is shown in Fig. 1. In each instance, the diagnosis was suggested on clinical grounds and was confirmed by demonstration of a deficiency or complete absence of galactose-1-phosphate uridyl transferase in the erythrocytes of the 35 living patients. In the remaining 10 cases, the diagnosis was based upon the usual clinical and laboratory findings, but not confirmed enzymatically. In addition, 48 of the 52 parents tested were found to have galactose-1-phosphate uridyl transferase activity in the heterozygote range.[3] The 47 unaffected siblings in these families were studied in a similar manner.

Galactose-1-phosphate uridyl transferase

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was assayed by two different methods: (1) The modified UDPG (uridine diphosphoglucose) consumption test described by Bretthauer and co-workers[4] and (2) the oxygen uptake method of Kirkman and Bynum[5] as modified in our laboratory.[6] The galactose-1-phosphate was determined by the procedure of Kirkman and Maxwell,[7] and galactose identified in the urine by means of paper partition chromatography.

One of three staff psychologists evaluated the intelligence of each patient by the Stanford-Binet form L.M. for older children and the Cattell Infant Intelligence Scale and the Vineland Social Maturity Scale for younger children. For 2 children with severe visual defects, the Maxfield Buchholz modification for blind children of the Vineland Social Maturity Scale was used.

RESULTS

Vital statistics.

Age distribution. The dates of birth of the galactosemic children and their unaffected siblings are given in Fig. 2. It is apparent that there is no significant difference in the age distribution of the two groups. Somewhat disturbing is the fact that, despite the more widespread knowledge and availability of lactose-free substitutes, the number of deaths in this small group of cases has not decreased within the last 5 years. This would indicate either that irreversible damage occurs prior to birth or that the diagnosis is still not made early enough.

Sex of affected individuals. There were 31 males and 14 females affected. If one compares this distribution with the ratio of 1.06 males to each female among the normal white population at birth, the excess of males is significant (χ^2 [1 d.f.*] = 6.61; p = 0.01). Of the unaffected siblings, there were 28 males and 19 females, an excess which is of no significance. There was a total of 7 reported miscarriages in these families, which is still not sufficient to account for the low incidence of affected females.[3]

*Degree of freedom.

Birth weight of affected children. The mean birth weight of all galactosemic children is 0.30 kilogram (10.5 ounces) less than that of their unaffected siblings, a difference which is significant at the 0.05 level (Table I). Furthermore, the galactosemic children who failed to survive weighed on the average 0.42 kilogram (14.8 ounces) less than the unaffected siblings, a difference again significant at the 0.05 level. Although the numbers of cases studied so far are still small, there is a suggestion that there may be a prenatal influence in galactosemia just as has been shown in cystic fibrosis of the pancreas[8] and in diabetes mellitus.[9]

Other findings. No significant differences in the parental age at the time of birth, the birth order, or the length of gestation are found between the affected and unaffected individuals (Table I).

Presenting clinical symptoms.

Hepatic symptoms. The presenting symptoms in the 45 cases are given in Table II. Jaundice occurred in 26 cases (58 per cent).

Fig. 1. Summary of data on 27 galactosemic families.

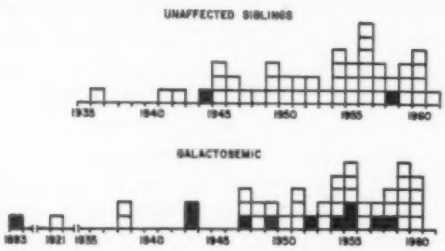

Fig. 2. Chart showing year of birth of galactosemic children and unaffected siblings. The individuals who have died are shown in black.

It is notable that, in a review of the hospital records, jaundice was either given as a very prominent presenting symptom or was not recorded at all. Hepatomegaly occurred in 31 cases (69 per cent), splenomegaly in 17 cases (38 per cent), and ascites in 11 cases (25 per cent). Surprisingly, ascites did not necessarily indicate a poor prognosis and may have, in certain instances, reflected the hypoproteinemia of malnutrition rather than liver failure.

Gastrointestinal symptoms. Failure to thrive in early infancy was a feature in many cases. Vomiting was present in 25 cases (55 per cent), weight loss in 21 (47 per cent), and diarrhea in 15 (33 per cent). All of these changes were rapidly corrected after the patients were placed on a lactose-free diet.

Eye findings. Cataracts were first noted from 7 days to 6 months of age in 21 cases (47 per cent). If cataracts did not appear by the age of 6 months, they were unlikely to develop thereafter. The cataracts were irreversible even with a lactose-free diet unless the patient was started on treatment at a very early age. In addition, there were 2 patients who developed retinal detachment, one of whom suffered bilateral intraocular hemorrhages resulting in total blindness.

Other features. In 12 cases (26 per cent), anemia was a prominent feature. The anemia in galactosemia appears to be partly hemolytic in nature. In several patients, we have noted a sudden drop in hemoglobin concentration without bleeding, followed by an increased reticulocyte count. Also, hemolysis was observed in vitro when the patient's red cells were incubated in a medium containing galactose. In addition, 2 patients developed purpura. Convulsions were noted in 5 cases (11 per cent), in one accompanied by hypoglycemia. Another patient had hypoglycemia without convulsions.

In addition to the clinical features, a reducing substance was detected in the urine of 28 of the patients initially (62 per cent).

Asymptomatic cases. Of great interest is the fact that 3 of the galactosemic persons were completely asymptomatic and the disease was discovered in the course of genetic studies within the family. Patient J-6 has been tested on four separate occasions and has always had enzyme levels in the homozygous range. He is now 1 year old and has been on cow's milk ever since birth without showing any of the clinical manifestations of the disease. Patient P-4 is a 40-year-old female office worker who has been well all her life. An excess of galactose-1-phosphate has been found in her erythrocytes and no galactose-1-phosphate uridyl transferase can be demonstrated by either method. Galactosemia was diagnosed in Patient I-5 at the age of 63 after he had led a successful life as a business executive. In the years immediately preceding his death, he had suffered from cirrhosis of the liver and cataracts. The

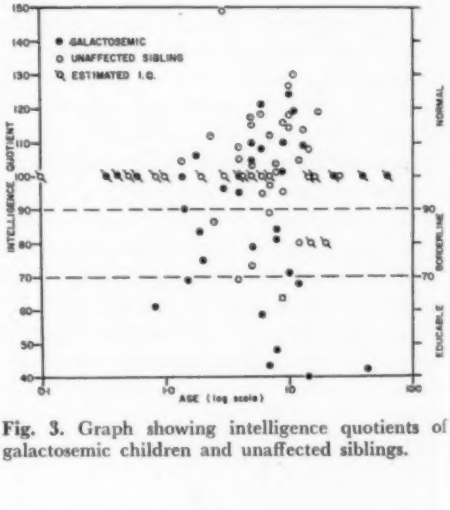

Fig. 3. Graph showing intelligence quotients of galactosemic children and unaffected siblings.

Table I. Summary of general findings among galactosemic children and unaffected siblings

	Galactosemic						Unaffected siblings	
	Alive		Dead		Total			
	No.	Mean S.D.	No.	Mean S.D.	No.	Mean S.D.	No.	Mean S.D.
Father's age (years)	32	30.5 ± 6.4	10	27.7 ± 5.2	42	29.8 ± 6.6	48	28.3 ± 6.2
Mother's age (years)	32	28.0 ± 6.3	10	22.8 ± 5.6	42	26.7 ± 6.8	48	25.8 ± 4.6
Birth order (No.)	33	2.66 ± 2.01	12	2.58 ± 2.44	45	2.63 ± 2.15	48	3.37 ± 2.80
Weight at birth (kilograms)	32	3.24 ± 0.70	9	3.08 ± 0.38	41	3.20 ± 0.67	44	3.50 ± 0.67
Gestation length (days)	32	273 ± 13	10	274 ± 19	42	273 ± 14	44	277 ± 6

diagnosis was not considered, however, until his grandchildren were found to have the disease. Specific investigations then disclosed the absence of galactose-1-phosphate uridyl transferase in the erythrocytes on three separate occasions, an abnormal galactose tolerance test, and the presence of galactose in the urine.

Postmortem findings. In the present series, 12 of the 45 patients (27 per cent) have died. With one exception, all of the deaths occurred between the ages of 6 days and 4½ months following a typical course of jaundice, hepatomegaly, weight loss, vomiting, and diarrhea. The other patient died at 66 years of age after a prolonged course of hepatic failure characterized by cirrhosis, ascites, and hepatic coma. Postmortem examinations were performed in 7 cases. In all instances, the predominant finding was cirrhosis of the liver associated with ascites and portal hypertension.

Present clinical status.

Physical health. Once past early infancy, patients with galactosemia generally enjoy good health. The gastrointestinal symptoms almost completely disappear, if they are kept on a lactose-restricted diet. Most of the patients will admit that after drinking a glass of milk, they suffer abdominal discomfort and some will develop diarrhea.

Physical examination shows that 14 of the 29 affected children are more than one standard deviation below normal for weight, while only 8 of the 40 unaffected siblings fall into that category, a difference which is significant (χ^2 [1 d.f.] = 6.3; p = < .02). Similarly, 13 of the 29 affected children are more than one standard deviation below normal for height, while only 7 of the 40 unaffected siblings fall into that group, and this difference is also significant (χ^2 [1 d.f.] = 7.0; p = 0.01). In addition, among the 33 living patients, 5 (15 per cent) showed persistent hepatomegaly and 11 (33 per cent) had cataracts, which are presumed to be permanent.

Mental status. The results of the psychological tests performed on the galactosemic patients and their unaffected siblings are shown in Fig. 3. Among the 34 with galactosemia, 20 patients (60 per cent) were in the normal range (I.Q. over 90), 7 (20 per cent) were in the borderline range (I.Q. 70 to 90); and 7 (20 per cent) were in the retarded but educable range (I.Q. 40 to 70). Among the 46 unaffected siblings, 39 individuals (85 per cent) were in the normal, 5 (11 per cent) in the borderline, and 2 (4 per cent) in the educable range. This demonstrates that although only a small segment of galactosemic persons are mentally deficient, the incidence is significantly greater than among their unaffected siblings (χ^2 [2 d.f.] = 25.8; p < .001).

Biochemical findings. The biochemical findings in the galactosemic patients and their unaffected siblings is given in Table III.

Galactose-1-phosphate uridyl transferase. With one exception, all of the *galactose-1-phosphate uridyl transferase* levels in the galactosemic patients fell within the homozygote range. In case H-1, there was no

Table II. Summary of presenting symptoms and present clinical status. Symptoms still present are circled

Case No.	*Jaundice*	*Hepato-megaly*	*Spleno-megaly*	*Ascites*	*Vomit-ing*	*Diarrhea*	*Weight loss*	*Cataracts*	*Anemia*
Alive									
A-1				+	+	+	+	⊕	
B-5	+	+	+		+		+		+
C-2		+	+	+					+
D-2	+	⊕	+		+	+	+	⊕	+
E-3	+	+	+				+	⊕	+
F-3	+	+	+				+		+
G-2		+		+	+	+	+		+
H-1	+	⊕	⊕				−	+	
I-1					+	+		+	
I-3		+			+	+	+	+	
J-5	+	+			+	+			
J-6									
K-2		+	+		+	+	+	⊕	
K-3	+				+		+		
M-1		+						⊕	+
N-3				+	+			⊕	
O-1		+				+		+	+
P-1	+	+						⊕	+
P-3	+	+	+				+	⊕	
P-4									
Q-1	+	+		+				⊕	+
R-7		⊕	+		+	+	+		+
S-1	+				+			⊕	
S-3	+	+	+					⊕	
T-1	+				+		+		
T-2	+								
U-3	+				+		+		
V-3	+	+		+	+	⊕	−		
W-3		⊕	+				+	+	
X-1	+	+			+			⊕	
Z-6	+	+	+	+	+		+	+	
AA-3									
AA-5									
Dead									
B-2	+	+	+		+	+	+		
B-8	+	+	+						
I-1	+	+	+		+		+		
I-5		⊕		⊕				⊕	
J-1		−							
L-1		+			+	+	+		
R-2		+	+		+	+	+		
V-1	+	+							
Y-1	+	+	+	+	+	+		+	
Y-2	+	+	+		+	+			
Z-1	+			+	+				
Z-3	+	+		+	+			+	+
Total	26	31	17	11	25	15	21	21	12

Convulsions	Hypoglycemia	Mental deficiency	Reducing substance	Diagnosed enzymatically	Age when last seen (years)	
						Other factors
			+	+	23	Detached retina
+		⊕	+	+	7	Listeria meningitis 1 week
		⊕	+	+	8	
		⊕	+	+	23	
			+	+	5	
			+	+	5	
			+	+	5	
			+	+	7/12	
+	+		+	+	12	Megacolon
			+	+	7	Osteoporosis
		⊕	+	+	2	All unaffected sibs mentally deficient
		⊕	0	+	1	
			+	+	14	
			+	+	10	
+		⊕	+	+	10	
+		⊕	+	+	10	CNS damage
			+	+	1	
				+	12	
				+	6	
				+	40	
		⊕	+	+	5	CNS damage
		⊕	+	+	1 9/12	
+		⊕		+	8	
				+	4	
			+	+	9	Asthma
		⊕	+	+	8	
			+	+	4/12	
		⊕	+	+	2	Purpura
			+	+	7/12	Retinal detachment
			+	+	2	Retinal detachment, blind, intraocular hemorrhage
			+	+	2	
			+	+	14	
	–	⊕		+	4	
					Age at death	*Postmortem findings*
					3 weeks	—
					11 days	—
					2 weeks	—
			+	+	66 years	Cirrhosis, portal hypertension
					5 days	—
	+				4½ months	Hepatomegaly, cerebral edema
					3 weeks	Fatty liver, atelectasis, pneumonitis
					6 days	Cirrhosis, severe purpura
			+	+	4 weeks	Cirrhosis with ascites
					9 days	—
		+			4 months	Hepatic cirrhosis
					7 weeks	Hepatic cirrhosis
5	2	14	28	35		

enzyme by the oxygen uptake method, but there was a UDPG consumption of 5.3 μM per hour per 0.6 Gm. hemoglobin, which persisted upon retesting. As the child had typical clinical symptoms, showed galactose in the urine, and both parents were found to be heterozygotes, the child was classified as having galactosemia. It could well be that with better definition of the alternate pathways for the degradation of galactose[11, 12] inconsistencies such as this will be classifiable.

Among the unaffected siblings, 23 showed enzyme levels in the heterozygote range, 11 showed enzyme levels in the normal range, and the remainder were either not classifiable[3] or not studied. This fits well with the concept of an autosomal recessive gene in which two thirds of the unaffected siblings are heterozygotes.

Galactose in the urine. In random samples of urine, galactose was demonstrated in 13 of 22 galactosemic patients (59 per cent) tested. On the basis of clinical history, there appeared to be no close correlation between dietary control and presence of galactose in the urine. Of those spilling galactose, 7 were in excellent, 5 were in fair, and 1 was in poor control, while, of those free from galactose, 5 were in excellent, 2 were in fair, and 2 were either in poor control or on no diet at all.

Galactose-1-phosphate levels. There was an accumulation of galactose-1-phosphate in the erythrocytes in most of the patients with galactosemia. Despite the fact that the determinations were based on a single sample in each instance, there was a fair degree of correlation between levels in the blood and dietary control. The two highest levels occurred in one patient whose diet was not restricted (AA-3) and in another just prior to the initiation of therapy (R-7).

Treatment. Data on the effect of therapy are presented in Table IV.

Forms of treatment. In the present series, 30 of the 33 living patients are on some type of lactose or galactose restriction. Among those under treatment, all 30 avoid liquid milk and 17 make a real effort to avoid all milk-containing foods such as bread, cake, puddings, etc. Various types of milk substitutes have been used during infancy. Nine patients were treated with Nutramigen, 6 with Mull-Soy, 3 with Sobee, and 4 with meat-base formula.

The dietary control of all of the 33 galactosemic children together with Patient I-5 who died at 66 years of age has been arbitrarily classified as being: (1) excellent, where the family makes every effort to avoid milk-containing foods and where no exceptions are allowed; (2) fair, where milk-containing foods are generally avoided, but occasional lapses occur, especially on holidays and birthdays; (3) poor, where no effort is made to restrict the diet, except avoiding milk. We realize this type of classification is crude; but since in every instance a home visit was carried out and the problem discussed in detail with the family, it probably reflects a fairly accurate impression about the degree of control. Using these criteria, 15 were in excellent, 11 were in fair, and 4 were in poor control; 3 were on no dietary control.

Effect of diet upon symptoms. There appears to be no question that the restriction of lactose in the diet has resulted in a disappearance of vomiting, diarrhea, and a reversal of the trend toward weight loss. In most instances, jaundice, hepatomegaly, splenomegaly, and ascites have diminished or completely disappeared. However, cataracts have persisted in 13 of the 20 patients and many of these have ultimately required extraction. Thus, in this area, the lactose-free regimen has not been entirely effective.

Effect of diet upon intelligence. Perhaps the most critical question is whether the lactose-restricted diet has any influence upon the ultimate mental development of the child with galactosemia. This will be considered first in terms of when the diet was started and then of the degree of control which was achieved.

The age at which the diet was started is given in Table V, and seems to have relatively little influence upon the I.Q. (χ^2 [6 d.f.] = 5.2; p = 0.5). This is quite in contrast to the impression gained in phenyl-

Table III. Summary of chemical observations

Galactosemic					Unaffected		
	Gal.-1-P uridyl transferase*		Galactose			Gal.-1-P uridyl transferase*	
No.	UDPG (μM/hr./0.6 Gm. hgb.)	O_2 uptake (μl/hr./ Gm. hgb.)	Gal.-1-P (mg.%)	In urine	No.	UDPG (μM/hr./0.6 Gm. hgb.)	O_2 uptake (μl/hr./Gm. hgb.)
A-1	3.0	0	0	+	B-1	8.1	254
B-5	0	0	8.8	+	B-3	7.0	189
C-2	0	32	13.0	0	B-4	8.4	97
D-2	0	32	19.2		B-6	11.9	243
E-3	0	0	2.1	+	B-7	9.7	237
F-3	0	0	5.7	+	B-10	14.7	348
G-2	—	0	—		B-11	9.7	289
H-1	5.3	12	—		C-1	6.3	70
I-1	1.2	0	9.9	0	D-1	11.8	299
I-3	2.0	0	2.1	+	D-3	15.4	588
I-5	0.9†	0	—		D-4	8.5	144
J-5	0	0	12.1	+	E-1	4.9	190
J-6	0	22	—	0	E-2	5.4	168
K-2	0	0	7.3	+	E-4	3.3	94
K-3	0	0	3.6	0	F-2	6.6	131
M-1	0	0	—		G-1	2.4	481
N-3	0	0	4.5	+	G-3	7.7	693
O-1	0	0	14.0	0	G-4	—	180
P-1	3.5	19	—		I-2	13.8	—
P-3	3.6	0	—		I-4	7.5	—
P-4	0	0	6.0	0	J-2	4.1	116
Q-1	0	0	8.3	0	J-3	4.2	79
R-7	0	0	29.1	+	J-4	—	270
S-1	0†	—	—		L-2	13.5	—
S-3	1.1†	—	—		M-2	7.6	—
T-1	0	0	3.6	+	M-3	9.1	62
T-2	2.6	14	8.1	0	N-1	9.2	174
U-3	1.7	0	10.9		N-2	14.1	357
V-3	0	0	—	+	P-2	5.5	220
W-3	0.1	0	—	+	R-1	4.4	—
X-1	0	0	13.5	0	R-3	3.0	—
Y-1	0†	—	—		R-4	8.3	—
Z-6	0.4	0	—	+	R-5	8.7	—
AA-3	0	0	23.9		R-6	—	—
AA-5	0‡	—	—		S-2	—	—
					U-1	5.7	75
					V-1	1.9	0
					W-1	6.7	138
					W-2	4.3	188
					Y-3	7.1	157
					Z-2	11.1	—
					Z-4	7.5	—
					Z-5	5.5	—
					AA-1	—	—
					AA-2	—	—

*Values are as follows:

UDPG (μM/hr./0.6 Gm. hgb.)	O_2 uptake (μl/hr./Gm. hgb.)
Controls	
15.05 ± 3.20	358 ± 100
Heterozygotes	
7.85 ± 2.57	235 ± 103
Homozygotes	
1.19 ± 2.01	20 ± 32

†Performed by the method of Anderson and associates.[39]

‡Performed in another laboratory.

Table IV. Summary of dietary control

	Diet							*Control*			
No.	*Age started*	*Avoids milk*	*Avoids all milk foods*	*Nutramigen*	*Mull-Soy*	*Sobee*	*Meat-base formula*	*Excellent*	*Fair*	*Poor*	*No treatment*
A-1	10/12	+			+	+			+		
B-8	9 days	+								+	
C-2	1/12	+	+		+			+			
D-2	6/12	+				+				+	
E-3	5/12	+	+				+	+			
F-3	4/12	+		+					+		
G-2	5/12	+							+		
H-1	2/12	+	+	+				+			
I-1	9	+	+					+			
I-3	5	+	+					+			
J-5	1/12	+		+	+			+			
J-6											+
K-2	6/12	+		+					+		
K-3	3 days	+		+				+			
M-1	2/12	+			+					+	
N-3	2/12	+		+					+		
O-1	2/12	+	+				+	+			
P-1	4/12	+							+		
P-3	2/12	+							+		
P-4											+
Q-1	6/12	+								+	
R-7	1-6/12	+	+				+	+			
S-1	1-6/12	+	+						+		
S-3	2/12	+	+						+		
T-1	4/12	+	+	+					+		
T-2	1/12	+	+	+					+		
U-3	4/12	+	+			+	+	+			
V-3	2/12	+	+		+			+			
W-3	4/12	+	+		+			+			
X-1	1/12	+	+					+			
Z-6	6/12	+	+	+				+			
AA-3											+
AA-5	6/12	+	+					+			
Total 33		30	17	9	6	3	4	15	11	4	3

ketonuria where the age at onset of dietary control is of prime importance.[14]

The degree of dietary control and the I.Q. are given in Table VI. Here, the children who had fair or excellent dietary control appear to do very much better than those who have had poor dietary control or have had no dietary restriction at all (χ^2 [6 d.f.] = 15.4; p = 0.02).

DISCUSSION

In viewing the 45 cases of galactosemia as a whole, we have been able to get a composite picture of the disease which has hitherto been lacking from individual case reports.[17] One is impressed by the variability in the clinical manifestations, and the ability of children, after early infancy, to lead a relatively normal existence.

The present study has revealed a number of findings which have not previously been emphasized. First, the disease process appears to occur before birth. Evidence for this can

Table V. Effect upon intelligence of age at which lactose-free diet was started

	Intelligence quotient			
Age	*90+*	*70-89*	*40-69*	*Total*
Under 6 months	12	6	2	20
6 months to 1 year	4	1	1	6
Over 1 year	3	0	2	5
Total	19	7	5	31

χ^2 (6 d.f.) = 5.2; p = 0.5.

be found from three different directions: (1) there is an excessive accumulation of galactose-1-phosphate in the cord blood of affected children,[18] (2) the affected children weigh on the average 0.30 kilogram less than their unaffected siblings, with an even larger discrepancy among those who failed to survive, and (3) some of the galactosemic children already show signs of cataracts and cirrhosis of the liver at birth, changes which do not appear to be reversible by means of a lactose-free diet. This would lead us to believe that, as in hypophosphatasia, some of the changes in galactosemia take place in utero, and that, unlike the situation in phenylketonuria, immediate postnatal therapy may not be sufficient to reverse all of the sequelae of the disease process, and a few babies will be expected to succumb.

Second, there appears to be an excessive number of affected males as compared with affected females. In a recent paper, Hugh-Jones and co-workers[17] noted that, in all the published cases in the literature, there were 66 males to 40 females. If data from the present study were added to those figures, and duplicate cases excluded, there would be a total of 91 males as compared with 44 females, a difference which would become highly significant (χ^2 [1 d.f.] = 13.85; $p < 0.001$). The deficiency of affected females could result either from: (1) the condition being frequently lethal in females, possibly at an early stage of cell division, or (2) the condition frequently not being diagnosed in affected females because it is clinically milder in the female. The latter might in some manner be related to the progesterone effect on galactose metabolism reported by Pesch and his collaborators.[19] In any event, the lower incidence of galactosemia in females is a new observation and remains one of the major unsolved problems of this disease.

Third, galactosemia appears to be primarily a disease of early infancy. In our cases, the major clinical manifestations appeared within the first few weeks of life. These can be subdivided into those involving (1) the liver, including jaundice, hepatomegaly, splenomegaly, and ascites; (2) the gastrointestinal tract, including vomiting, diarrhea, and weight loss; and (3) the eyes, as shown by cataracts and intraocular hemorrhages. While the cataracts could well be explained on the basis of accumulation of galactose-1-phosphate within the lens capsule[20] and the inhibition of lenticular glucose-6-phosphate dehydrogenase by galactose-1-phosphate,[21] the cause for the latter is unknown. Death generally results from liver failure. The mechanism that brings this about is undoubtedly complex and metabolic in nature, since the condition of infants with cirrhosis of the liver without galactosemia does not deteriorate so rapidly.

The affected individual who survives the period of early infancy is frequently indistinguishable from a normal person. In a few instances hepatomegaly still persists, and certainly cataracts provide a vital clue. However, unless the individual is consuming a great deal of milk, which is somewhat unusual in the average adult, reducing substance is not a regular feature of the disease. In our series, only 13 of 22 patients were found to have galactose in urine specimens obtained at random. Since hypoglycemic episodes have been known to occur following a galactose tolerance test, this means that a definite diagnosis can be established only by the direct measurement of galactose-1-phosphate uridyl transferase in the red cells. The relative lack of acute symptoms in the older patients could be explained in two ways: (1) the actual intake of galactose in terms of body size or weight decreases with age, (2) the lack of galactose-1-phosphate uridyl transferase is compensated for by at least

Table VI. Effect of dietary control upon intelligence

	Intelligence quotient			
Dietary control	*90+*	*70-89*	*40-69*	*Total*
Excellent	10	4	1	15
Fair	8	2	1	11
Poor or none	2	1	5	8
	20	7	7	34

χ^2 (6 d.f.) = 15.4; p = 0.02.

two alternate pathways. Isselbacher[11] has shown that the galactose can be metabolized by UDP-galactose pyrophosphorylase. This enzyme has a very low activity in fetal and neonatal tissue, but increases in activity with age. Recently, Inouye and Hsia[12] have demonstrated the conversion of galactose-1-phosphate to galactose-6-phosphate in galactosemic cells and, since the latter compound can be removed by its dehydrogenase, this may prove to be yet another pathway for the removal of galactose in the older individual.

Perhaps the most unexpected finding of the present study is that nearly two thirds of the children were found to have normal intelligence, even though the child, in some instances, never had been treated or had been placed on a lactose-free diet at a relatively late age. It is apparent that the meager amount of information we have on the degree of dietary control in each case is insufficient to draw any definite conclusions as to its significance in mental development. However, it appears that the age when the diet was started is not as critically important as in phenylketonuria, although the degree of control may be of some importance. With the availability of a relatively simple technique for the measurement of galactose-1-phosphate,[7] it should be possible to follow all patients with galactosemia more objectively in the future. From the viewpoint of those interested in the etiology of mental deficiency, the variability in the distribution of the intelligence quotients in what appears to be a single metabolic defect offers a particular challenge. Perhaps in this body of data lies the answer to the role of galactose in the development of the metabolic processes of the brain.

Some of the data on the following patients have previously been recorded: C-2,[15] G-2,[16] I-1, I-5,[17] and Z-6.[18] We wish to express our appreciation to the following physicians for referring patients to us for this study: Drs. Fred Allen, Sr., Clark Fraser, J. C. Rathbun, Norman Pokorny, L. Welcher, Alvah L. Newcomb, Minnie Frank, Louis Bachrach, Carolyn McCue, Edward Stabins, Samuel Foman, Richard Goldbloom, Eugene McEnery, F. L. Phillips, J. Darrell Smith, Ira Rosenthal, Malcolm Holliday, Eugene Diamond, Joseph Christian, and Alfred Eckstein; and to the following members of the Genetic Clinic for their help: William Rowley, M.D., Margaret O'Flynn, M.B., Ch.B., Smilja Jakovcic, M.D., Sumati Nair, M.D., Tohru Inouye, Ph.D., Morag Cummings, M.B., Ch.B., June Cosbey, M.F.A., and Marina Barrientos. The psychological tests were performed by Drs. Jack Riseman, David Emeson, and Paul Frappier.

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Adrenocortical and adrenocorticotropic function in children

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THIS report deals with an evaluation of adrenocortical function and adrenocorticotropic (ACTH) reserve in children. Appropriate tests have been adapted for this purpose and applied to a group of normal children and several with disorders of the adrenal or pituitary glands.

In order to assess the function of the adrenal cortex in children, the corticosteroids in blood and urine may be measured and the levels compared with reliable normal standards. The test is enhanced by determining the adrenocortical reserve following the administration of ACTH, under which conditions a predictable rise of corticosteroids would occur if the adrenal cortex were "normally" responsive. This inclusive approach has been documented by other workers[1-4]; we have employed techniques based on these reports. Recently, we have sought to simplify the methods in the light of newer developments and to standardize them in normal children.

On the other hand, the assessment of hypophyseal function must be approached differently. In our studies, only the secretion of ACTH (among the several hormones of the anterior pituitary) has been considered. Ideally, it would be desirable to measure the levels of ACTH in the blood directly, but the methods designed for this purpose remain tools for research, at present, and do not lend themselves universally to clinical study. It is possible to evaluate pituitary function, indirectly, by considering "target organ" performance as a reflection of adequate tropic influence, but the limitations of this classic device are well recognized.[5] The introduction of SU-4885* (2-methyl-1, 2-bis(3-pyridyl)-1-propanone) as a means of provoking endogenous ACTH release has afforded a convenient tool for the stated purpose.[6] The administration of SU-4885 to normal subjects produces a greater secretion and re-

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lease of ACTH, which reaction, in turn, produces alterations in adrenocortical function which may be measured readily. The mechanism is detailed below. The results obtained with this test in healthy children and in those with presumed or proved pituitary disease are presented.

MATERIALS AND METHODS

The adrenocortical response to a standard dose of intramuscularly administered ACTH was studied in 25 normal children, 1 week to 16 years of age, in 3 children with adrenal disease, and in 5 who had previously received pharmacologic doses of glucocorticoids.

SU-4885 was administered to 10 normal children, to 4 children presumed to have anterior pituitary deficiency, to 3 children with diabetes insipidus, and to 2 with acquired hypothyroidism. Adrenocortical responsiveness was measured by determining the levels of plasma corticosteroids,[7] before and 4 hours after an intramuscular dose of ACTH-Gel (30 mg. per square meter). All tests were begun at 8.00 A.M. Endogenous adrenocorticotropic reserve was tested by measuring urinary corticosteroids before, during, and after the administration of SU-4885 (500 mg. orally every 4 hours for 6 doses). The levels of steroids were measured in urine collected the day before, the same day, and the day following the administration of this drug. Although the method was essentially that of Silber and Porter,[7] certain modifications were introduced. Aliquots of urine from each collection were examined in duplicate; each was washed with petroleum ether. Only one specimen of urine of each pair was washed with 2½ volumes of carbon tetrachloride. The latter solvent extracts most of the metabolites of 11-desoxycortisol (Substance S), but few of those deriving from cortisol (Compound F). Thereafter the procedure remains as described. The differences between the Porter-Silber chromogens of the duplicate specimens then represents the metabolites of S rather than of F. This modification should be beneficial since SU-4885, in exerting its anticipated effect, should lead to the production of relatively large amounts of S.

*Supplied by the Ciba Pharmaceutical Company, Inc.

RESULTS

Adrenocortical responsiveness to exogenous ACTH is summarized in Table I. Among the 25 healthy children there were no significant variations due to age or sex within the ranges studied. In all normal children, the minimal level of blood corticosteroids after stimulation was always higher than the respective base line and varied between 29.0 and 59.9 (mean 35.7) μg per cent. Only the free, unconjugated corticosteroids were measured. As noted by others,[2, 8-10] the level of corticosteroids achieved following ACTH was independent of the level prior to stimulation. One child with the adrenogenital syndrome due to congenital adrenal hyperplasia had an original level of corticosteroids within the normal range, but there was no response to ACTH, a result anticipated from previous studies in this laboratory.[11] Two children with Addison's disease exhibited low levels of blood corticosteroids before and after ACTH. All of the 5 children previously treated with glucocorticoids demonstrated impaired response. This has been reported by others.[12, 13] Our data are insufficient to allow conclusions which relate the response to the duration of prior treatment with corticosteroids or to the interval of withdrawal.

The responses to the administration of SU-4885 are shown in Table II. Among 10 normal children, the rise of the total urinary corticosteroids (a consequence of their own endogenous ACTH) was never less than 2½ times the control level and was as great as twelvefold. The greatest level was more often achieved on the day following administration of the drug. The appearance of Substance S, a direct result of the inhibition of 11-β-hydroxylase, accompanied the rise in corticosteroids and constituted 49 to 92 per cent of the total. The mean values and their standard deviations are shown in Table II. Four children of significantly diminished stature, with delayed sexual and osseous matura-

Table I. Adrenocortical responsiveness to exogenous ACTH determined by blood tests

Patient No.	*Blood corticosteroids (μg %)*		*Comments*
	Control	*4 hours after ACTH*	
25 Controls			
Mean	9.3	35.7	Healthy
S.D.	± 5.2	± 6.7	
1	5.4	5.0	Untreated congenital adrenal hyperplasia
2	2.1	0.9	Addison's disease
3	0	0	Addison's disease
4	3.8	12.1	Corticosteroid therapy for 5 days. Off ½ day (asthma)
5	5.5	13.6	Corticosteroid therapy for 6 months. Off 2 days (rheumatoid arthritis)
6	9.1	18.6	Corticosteroid therapy for 1 year. Off 8 weeks (rheumatoid arthritis)
7	0.8	4.2	Corticosteroid therapy for 16 months. Off 12 days (rheumatoid arthritis)
8	4.1	5.3	Corticosteroid therapy for 2 years. Off ½ day (rheumatoid arthritis)

tion, and with clinical manifestations of anterior hypopituitarism were studied. Two cases (1 and 3), one with clear radiologic evidence of a large sellar tumor, responded in normal fashion, indicating adequate hypophyseal adrenocorticotropic function. The other 2 failed to demonstrate a significant response. However, subsequent administration of ACTH in the manner described earlier led to a normal response of blood corticosteroids in both of these children. Three children with well-documented diabetes insipidus responded normally, and, indeed, one of these (No. 2) attained a level beyond the standard deviation of the group of normal children. The two children with acquired hypothyroidism responded well and achieved levels notably greater than those in

Table II. Responsiveness to SU-4885 determined by urine tests

Patient (No.)	*Age (yrs.)*	*Urinary corticosteroids (mg. per 24 hrs.)*			*Urinary tetrahydro-S (%)*		*Comment*
		Control	*Day of SU-4885*	*Day after SU-4885*	*Day of SU-4885*	*Day after SU-4885*	
10 Controls	4-13						
Mean		1.1	3.1	4.3	60.2	58.2	
S.D.		± 0.68	± 1.3	± 1.6	± 16.0	± 15.8	
Hypopituitary							
1	6½	0.61	0.59	3.65	0	85	Idiopathic
2	13	1.4	0.3	0.3	0	0	Idiopathic
3	14½	1.2	2.2	5.3	61	83	Sellar tumor
4	16	0.17	0.32	0.30	34.7	10.7	Idiopathic
Diabetes insipidus							
1	3	0.8	0.9	2.6	27.4	56.2	Idiopathic
2	5	1.8	2.2	10.0	61.8	66.6	Idiopathic
3	14	0.3	1.37	3.10	67.9	76.2	Idiopathic
Acquired hypothyroidism							
1	6	1.8	9.9	16.4	78	92	
2	14½	1.5	2.6	8.3	66.3	72.8	

the normal group. Two children developed symptoms regarded as side effects of the drug; a single episode of emesis occurred in one, and headache with nausea in the other.

DISCUSSION

One phase of the study was to test adrenocortical response in children. It has become customary to do this by the administration of ACTH, which directly stimulates the gland. These investigations have incorporated the advantages of a single intramuscular injection of ACTH-Gel and the use of the relatively simple technique of measuring blood corticosteroids[7] as a test of adrenocortical responsiveness in children. Since our previous studies had employed more cumbersome methods,[11] it was first necessary to observe the response in a group of normal children. Under the conditions employed, normal children showed good responses which were easily demonstrated. The choice of a 4-hour interval was based on the observations of Eik-Nes and co-workers.[14] As anticipated, no response was obtained in 3 children with adrenocortical disease. The single subject with congenital adrenal hyperplasia revealed a normal base line level of corticosteroids, an observation in accord with the proposition considered elsewhere, namely, that many of these subjects may "compensate."[15] But the failure to produce a rise with exogenous ACTH suggests maximal endogenous ACTH stimulation and adrenocortical performance at the time of the test. The impaired response in children who received glucocorticoids indicates adrenal suppression with as little as 5 days of treatment; however, conclusive evidence on this point is lacking since the pretreatment response was not studied. Others[12, 16-18] have shown poor adrenal response to stress for variable periods following the administration of glucocorticoids, and it has also been suggested that hypophyseal function may be suppressed.[19-22]

The second facet of the study concerns the assessment of endogenous secretion of ACTH by the anterior pituitary gland of the child. It is obvious that the exogenous administration of this tropic hormone to test adrenal responsiveness yields no information concerning the integrity of the pituitary gland itself. To date, there have been many difficulties in assessing the blood levels of ACTH directly. Insulin-induced hypoglycemia has been used as an indirect measure of the function of the hypothalamic-pituitary-adrenal axis.[5, 23] Pyrogens have also been administered as a means of testing the latter system.[24, 25] The advent of SU-4885 has made possible the appraisal of endogenous adrenocorticotropic secretion and release.[6, 25-28] This drug interferes with the biosynthesis of cortisol and permits production of Substance S in its stead. Since the latter is not a physiologic "regulator" of the pituitary-adrenal system, greater amounts of ACTH are normally produced in an attempt to restore the levels of cortisol. During the course of these events, an increase of both S and F occurs, both of which substances are measured as urinary corticosteroids with the method employed in the study. Such a partition of S and F has been recommended previously.[28] The modification recommended here permits the differential estimation of S and F, as shown in Table II. Since both F and S are produced during the test, it is apparent that 11-hydroxylation is not completely inhibited. The degree to which this occurs may be a function of duration and dosage, a matter not considered in this study. Nonetheless, the dosages employed in this study were adequate to secure measurable changes in the urinary corticosteroids of children. Failure to find a rise in urinary corticosteroids following administration of SU-4885, as in the 2 children with clinical hypopituitarism (Cases 2 and 4), suggests deterioration of either hypophyseal or adrenocortical function. The 2 children who did not respond to SU-4885, when tested with exogenous ACTH, exhibited elevation of the blood corticosteroids which were well within normal limits. Hence, it may be concluded that their disturbance lay in the anterior pituitary gland. It may not be concluded that the deficiency involved secretions other than adrenocorticotropin, as based on the criteria applied, although the clinical picture implied

somatotropic and gonadotropic insufficiency. An isolated deficiency of adrenocorticotropin has been proposed by others.[29] The other 2 children with presumed anterior hypopituitarism (Cases 1 and 3), reacted to SU-4885 with adequate elevations of urinary corticosteroids despite the fact that one of them had radiologic evidence of organic sellar disease. Possibly other functions of the anterior hypophysis are amiss in the last 2 subjects; only the adrenocorticotropic aspects can be tested with this agent. It must also be conceded that the diagnosis of hypopituitarism in childhood is presumptive under conditions of available diagnostic methods. Three children with posterior hypophyseal dysfunction (diabetes insipidus) responded normally to SU-4885, and this indicates anterior pituitary integrity insofar as adrenocorticotropin is concerned. The somewhat exaggerated elevation of urinary corticosteroids in 2 children with hypothyroidism is difficult to explain. Is the hypophyseal adrenocorticotropic mechanism more sensitive in this disease? Rather, it may be that SU-4885 is more slowly metabolized in this disorder, and, therefore, exerts a greater action on the adrenal. This is suggested by the very high percentage of S in one of these hypothyroid subjects after the drug was administered.

A degree of caution is recommended in the use of SU-4885. Although mild reactions were noted in only 2 of our children, more severe ones have been described with higher doses in adults.[27]

CONCLUSIONS

1. The response of blood corticosteroids to the administration of intramuscular ACTH-Gel has been standardized in 25 normal children. The blood levels rose from a mean of 9.3 μg per cent to 35.7 μg per cent at 4 hours after the administration.

2. An impaired response to exogenous ACTH was noted in 3 children with adrenal disorders, and in 5 others who had previously been treated with glucocorticoids.

3. Adrenocorticotropic response was measured by the elevation of urinary corticosteroids following the administration of SU-4885 (2-methyl-1,2-bis(3-pyridy 1)-1-propanone). In 10 normal children the mean values of urinary corticosteroids prior to treatment were 1.1 mg. per day; after administration for a single 24-hour period, they were 3.1 mg. on the day of treatment and 4.3 mg. on the day following it. In all children tetrahydro-S was detectable in the urine in large amounts following administration of the drug.

4. Two children with presumed anterior hypopituitarism failed to respond to SU-4885, but did respond to ACTH. Two other children with presumed anterior hypopituitarism, 3 children with diabetes insipidus, and 2 with acquired hypothyroidism responded well to SU-4885. There was an exaggerated response in 2 children with hypothyroidism.

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Studies on submaxillary saliva in cystic fibrosis

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THE term cystic fibrosis represents a variety of symptoms resulting from an alteration in the normal physiologic function of a number of exocrine glands with the intensity of these functional alterations varying considerably from one patient to another. The abnormality of the tracheobronchial secretions represents the most serious disturbance and, thus, an understanding of the pathophysiologic alteration associated with the seromucous glands is essential for the organization of a firm basis for therapy. Published studies[1] from this laboratory delineated, to some extent, the inorganic and organic composition of the tracheobronchial secretions in this disease as compared with those obtained from patients with bronchiectasis. They pointed out quite clearly that there are many factors associated with the analysis of tracheobronchial secretions which very seriously hamper an accurate elucidation of the pathophysiologic alterations of these glands. First the secretions contain not only substances associated with glandular secretion, but also products of exogenous contamination, i.e., bacteria, white blood cells, products of their metabolism, and substances associated with the disintegration of such material. In addition, the amount of contamination is greater than that found in the secretion obtained from normal and bronchiectatic individuals. Second, especially in cystic fibrosis, the secretions accumulate and may be present in the bronchial tree for varying periods of time and, thus, subject to environmental factors, i.e., dehydration, which may alter their physicochemical characteristics.

It was because of such factors that our attention was drawn to the submaxillary gland. This gland produces a seromucoid secretion comparable, in some respects, to the secretion in the tracheobronchial tract. There is a known pathologic alteration which results in the obstruction of the ducts. Thus, submaxillary saliva offers a unique opportunity for analyzing a seromucoid secretion, in a dynamic state, from a pathologically affected gland containing no contaminants.

This initial report on submaxillary saliva in cystic fibrosis describes the collection method and attempts to characterize the ionic interrelationships that exist in this se-

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cretion under both physiologic and pharmacologic stimulation.

METHODS

Collection of submaxillary saliva, throughout these experiments, was accomplished through the utilization of individually constructed salivary segregators. Such appliances as described by Schneyer[2] were modified for submaxillary saliva collection from 12 children with cystic fibrosis and 12 control children (Fig. 1).*

In children, the collection of submaxillary saliva requires a period of training in which the child becomes accustomed to maintaining the tongue in a relaxed position without excessive movement and to maintaining a uniform psychological attitude toward the test. This necessitates selecting an intelligent child with good coordination. The period of training varies with the individual and may require several visits before uniform results are obtained. In order to obtain reproducible results, the collector must fit the child perfectly. This is difficult because of the rapid growth and movement of the teeth in children. Consequently, it is necessary for the dentist to fit the collector to the child at each collection of saliva. This necessitates complex adjustments to the apparatus, in many instances, from one collection period to another. In addition, the rapid growth and movement of the teeth in many cases required the making of entirely new collectors after a period of only several months. Experience has shown that it is absolutely essential that the collector fit perfectly in order to obtain reproducible and reliable results.

*The method utilized for making such appliances was as follows: At the patient's first visit, a complete explanation of the procedure was given and an impression taken and processed in dental stone. A wet piece of cellophane was then adapted over the lingual and occlusal portions of this model. A mass of self-curing acrylic was manipulated so as to form an impression tray covering the occlusal and lingual surfaces to the base of the tongue. Forty-five minutes prior to the second impression, 50 mg. of Banthine is administered to arrest salivary flow. The patient is acquainted with the feeling of the acrylic tray in position, and establishes a natural tongue position. An impression is taken with alginate on the custom tray while the tongue is in a natural relaxed position. The final impression is utilized to construct a model of gypsum casting investment material. A circle is then scored deeply into the model enclosing the two orifices of Wharton's ducts. Base plate wax is adapted to the occlusal and lingual surfaces and luted in place. A piece of No. 28 gauge wax is wrapped to a double thickness around a pencil lead about 1 inch long, and the tube is luted into place over the scored circle. This wax pattern of the collector is prepared and cast in aluminum. After it is cast, the pencil lead is drilled out, leaving a hollow tube. The collector is polished, fitted, and a Tygon tube attached.

COLLECTION PROCEDURES

Various procedures were utilized in collecting submaxillary saliva, depending on the requirements of the experiments. All collections were made at least 3 and usually 5 hours after ingestion of food. Reflex stimulation of submaxillary saliva was obtained with orange-flavored lozenges* placed on the tongue. As stimulation proceeded, submaxillary saliva flowed through the orifice in the collector, through the attached Tygon tubing into a graduated centrifuge tube fastened to the chin with tape. Tubes were exchanged at exactly 2 minute intervals in the samples collected for the analysis of flow, and of sodium, potassium, chloride, and nitrogen. Four consecutive 2 minute collections were made in each experiment. Samples collected for calcium analysis consisted of two consecutive accurately timed 5 ml. samples. In each sample obtained for calcium analysis, comparative determinations were also made on flow, sodium, potassium, chloride, and nitrogen.

The collection of submaxillary saliva for the determination of hydrogen ion concentration was accomplished in the following manner: Hypodermic syringes filled with 0.5 ml. of mineral oil were attached by means of adapters to the Tygon tubing connected to the collector. At 2 minute intervals, syringes were quickly exchanged and a stopper immediately placed on the tip of the syringe.

Experiments involving pharmacologic stimulation of submaxillary saliva were performed in an attempt to elicit greater magnification of potential differences. Parasympathomimetic stimulation was achieved with methacholine chloride in a dose of 0.1 mg. per kilogram injected subcutaneously. This dose, derived experimentally, represents the maximum dose that can be administered

*Life Savers, orange flavor.

Table I. Reflex stimulated submaxillary saliva

	Flow (ml./min.)	*Sodium (mEq./L.)*	*Potassium (mEq./L.)*	*Chloride (mEq./L.)*	*Calcium (mg. %)*	*Nitrogen (mg./ml.)*	*Hydrogen ion concentration, pH*	*Description*
Control	0.92 (0.55-1.94)	17.1 (5.8-35.9)	16.0 (11.5-21.7)	16.9 (7.8-35.8)	7.51 (5.77-9.22)	0.219 (0.153-0.416)	7.10 (6.90-7.20)	Clear
Cystic fibrosis	0.56 (0.16-0.98)	12.6 (5.3-30.2)	18.8 (11.3-22.8)	14.5 (10.1-32.4)	12.43 (8.02-14.38)	0.480 (0.330-0.778)	7.23 (6.90-7.30)	Turbid

without severe discomfort to the child with cystic fibrosis. Conditioning was also necessary during these experiments to prevent excessive movement of the tongue. Tubes were exchanged at 2 minute intervals for the duration of the experiment.

ANALYSIS

Sodium and potassium concentrations were determined with the use of a Baird flame photometer (Model DB-4) with lithium as the internal standard. All samples were run in duplicate with comparable standard measurements after each sample determination. Chlorides were determined by means of the Aminco-Cotlove chloride titrator with duplicate determinations on all samples; total nitrogen by a micro-Kjeldahl method on duplicate samples with comparative standards; hydrogen ion concentrations by means of a Beckman microelectrode apparatus with a Leeds and Northrup pH indicator. Such procedures allowed measurement of pH on volumes of 0.5 ml. of saliva. Standard buffers were compared after each determination. Calcium was analyzed in the following manner: Preliminary experiments indicated that the usual method of deproteination with trichloracetic acid was not sufficient to eliminate all interfering substances. Therefore, 3 ml. of submaxillary saliva was pipetted into a tared porcelain crucible. The samples were placed in an oven at 100° C. and dried to constant weight. The samples were then

Table II. Flow rate and sodium, potassium, and chloride concentrations in submaxillary saliva obtained by reflex and pharmacologic stimulation

	Flow (ml./min.)		*Sodium (mEq./L.)*		*Potassium (mEq./L.)*		*Chloride (mEq./L.)*	
Time (min.)	*Control*	*Cystic fibrosis*	*Control*	*Cystic fibrosis*	*Control*	*Cystic fibrosis*	*Control*	*Cystic fibrosis*
Reflex	0.9 (0.55-1.94)	0.6 (0.16-0.98)	17.1 (5.8-35.9)	12.6 (5.3-30.2)	16.0 (11.5-21.7)	18.8 (11.3-22.8)	16.9 (7.8-35.8)	14.5 (10.1-32.4)
Methacholine chloride, 0.1 mg. per kilogram subcutaneously								
2	2.2 (1.5-3.0)	1.9 (0.9-3.1)	46.6 (38-56.4)	49.1 (34.5-77.0)	17.0 (14.2-20.5)	17.6 (14.2-21.6)	41.4 (30.6-48.3)	46.3 (40.7-57.3)
4	2.8 (2.3-3.3)	2.2 (1.0-3.2)	68.6 (54.4-80.0)	71.0 (55.2-82.4)	12.4 (9.9-16.5)	14.9 (12.6-16.5)	44.4 (36.9-60.4)	54.9 (39.3-60.1)
6	2.5 (2.0-3.2)	1.8 (1.0-3.1)	65.2 (50-78)	69.0 (48.7-79.1)	12.1 (9.8-16.7)	11.1 (10.0-12.9)	37.5 (29.0-60.6)	49.6 (47-52.5)
8	2.1 (1.8-2.9)	1.4 (0.8-2.7)	60.7 (47-72.3)	63.6 (58.8-76.5)	10.7 (9.1-12.8)	11.2 (9.5-13.2)	31.6 (25.4-55.0)	39.9 (32.9-44.6)
10	1.7 (0.7-2.5)	0.92 (0.1-2.0)	49.3 (31.4-69.4)	42.4 (35.7-54.4)	11.6 (9.4-13.7)	12.4 (11.0-13.8)	21.8 (13.8-29.0)	26.5 (19-33.4)
12	1.2 (0.2-2.3)	0.45 (0.1-1.5)	37.9 (22.6-60.2)	29.5 (13.8-38.9)	14.4 (11.2-19.2)	14.5 (13.8-15.4)	16.6 (6.0-23.7)	16.4 (10.5-25.5)

ashed at 600° C. in a muffle furnace. The ash was carefully redissolved in water and a drop of concentrated nitric acid and brought up to a 6 ml. volume. Three 2 ml. aliquots were then prepared. Each 2 ml. sample was adjusted to pH 5.9 with dilute ammonium hydroxide.

Analysis of the SE samples was then accomplished by oxalate precipitation and titration with a standardized potassium permanganate solution as described by Rosenthal and co-workers.[3] Three determinations were made on each sample. In the case of the reflex stimulated samples, two crucibles were processed simultaneously for a total of six determinations per sample.

RESULTS

Table I represents the means and ranges of submaxillary saliva stimulated reflexly in patients with cystic fibrosis and control children. The data relating to flow and to sodium, potassium, and chloride content constitute the mean and ranges of 125 samples in each group, analyzed in duplicate. In addition, the data relating to calcium represent determinations on 48 samples; nitrogen, 80 samples; and pH, 24 samples for each group.

The flow of submaxillary saliva, although manifesting a substantial overlap, indicates a significant difference between the two groups with a mean of 0.92 ml. per minute for the control group as compared with a mean of 0.56 ml. per minute in the cystic fibrosis group. The sodium, potassium, and chloride values also exhibit considerable overlapping with no significant differences, the mean sodium and chloride concentrations being slightly lower in the cystic fibrosis group and the potassium concentration slightly higher. The mean values for sodium, potassium, and chloride in the two groups parallel the respective flow rates. A significant difference was observed between the calcium concentrations in the two groups. The control group manifested a mean value of 7.51 mg. per cent as compared with a mean of 12.43 mg. per cent in the cystic fibrosis group. The upper range limit in the control group represents one experiment and, thus, virtually no overlap is present between the two groups. Another significant difference between the two groups was found in the total nitrogen content of these secretions. As indicated in Table I, the mean nitrogen concentration is 0.219 mg. per milliliter in

Table III. Calcium and nitrogen concentrations and pH in submaxillary saliva obtained by reflex and pharmacologic stimulation

Time	*Calcium (mg. %)*		*Nitrogen (mg./ml.)*		*pH*	
(min.)	*Control*	*Cystic fibrosis*	*Control*	*Cystic fibrosis*	*Control*	*Cystic fibrosis*
Reflex	7.51 (5.77-9.22)	12.43 (8.02-14.38)	0.219 (0.153-0.416)	0.48 (0.33-0.78)	7.1 (6.9-7.2)	7.2 (6.9-7.3)
Methacholine chloride, 0.1 mg. per kilogram subcutaneously						
2	7.60 (5.9-8.7)	11.1 (10.6-12.2)	0.390 (0.25-0.53)	0.53 (0.31-0.74)	7.4 (7.2-7.5)	7.25 (7.0-7.4)
4	10.4 (6.2-11.4)	12.8 (10.5-13.4)	0.53 (0.22-0.92)	0.75 (0.61-0.92)	7.7 (7.6-7.8)	7.65 (7.6-7.7)
6	11.0 (7.5-14.1)	14.5 (12.9-16.1)	0.62 (0.46-1.03)	0.77 (0.44-1.20)	7.65 (7.5-7.7)	7.60 (7.5-7.7)
8	10.5 (7.6-12.9)	11.6 (10.8-12.6)	0.52 (0.33-0.83)	0.61 (0.49-0.71)	7.6 (7.3-7.7)	7.6 (7.5-7.7)
10	9.9 (6.3-11.7)	11.4 (11.0-12.1)	0.50 (0.31-0.69)	0.58 (0.44-0.86)	7.5 (7.2-7.7)	7.4 (7.2-7.5)
12	9.7 (6.0-12.2)	11.4 (9.8-12.9)	0.38 (0.30-0.53)	0.58 (0.48-0.70)	----	----

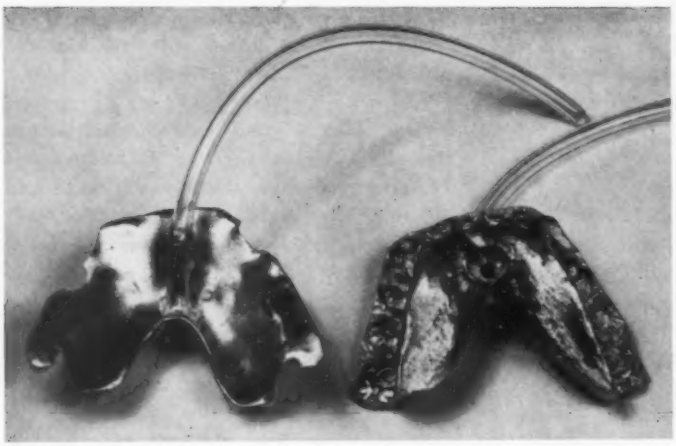

Fig. 1. Individually constructed aluminum appliance used for the collection of submaxillary saliva.

the control group as compared with 0.480 mg. per milliliter in the cystic fibrosis group. Some overlap was observed; however, the vast majority of the samples contained a higher nitrogen content in the cystic fibrosis submaxillary saliva than in the secretions obtained from control patients. No significant difference was observed in the hydrogen ion concentrations of the two groups.

One of the most striking differences observed between the submaxillary saliva collected under conditions of reflex stimulation from the two groups was the consistent presence of cloudiness in the saliva obtained from patients with cystic fibrosis as compared to the clear transparent saliva from the control children.

Table II represents the means and ranges of flow rate, sodium, potassium, and chloride concentration in submaxillary saliva, parasympathomimetically stimulated with methacholine chloride. This stimulation results in a bell-shaped response curve with a maximum effect in 4 to 6 minutes after injection.

The flow data indicate that the mean values, obtained under parasympathomimetic stimulation, are similar to the results obtained reflexly in that the cystic fibrosis group manifests a lower flow pattern than the control group. The pattern of values is fairly regular among the controls with the exception of variability produced near the terminal phase of the experiment, while it is generally irregular in the cystic fibrosis group. Sodium, potassium, and chloride values show fairly consistent patterns in both groups with comparable maximum values—this despite the irregularity of flow observed in the cystic fibrosis patients.

Table III represents the calcium, total nitrogen, and hydrogen ion concentrations. There is a significant difference in the mean calcium concentration between the two

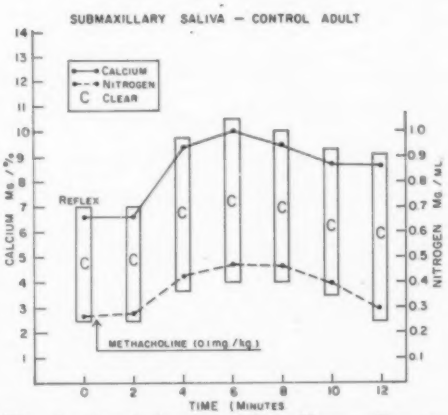

Fig. 2. Correlation of submaxillary saliva appearance and calcium and nitrogen concentrations under parasympathomimetic stimulation in an adult.

groups. The cystic fibrosis group has a definitely higher concentration of calcium than that of the controls, the overlap representing only a few samples. The nitrogen curves were similar to those for calcium. No significant difference was noted in the hydrogen ion concentration patterns.

The relationship of the cloudy solution to calcium and nitrogen values can best be described by the following groups of individual experiments. Fig. 2 represents simultaneous determinations of calcium and the nitrogen content under methacholine chloride stimulation in an adult. The dotted line indicates that the total nitrogen content rose about 0.2 to 0.3 mg. per milliliter at its maximum point and the calcium concentration rose approximately 4 mg. per cent. The saliva maintained a clear transparent appearance throughout the experiment. This was consistently observed in 8 adults. The sodium, potassium, and chloride curves for these experiments were comparable to the curves previously described.

Fig. 3 represents the calcium and nitrogen curves in a control child. The reflex stimulated sample was a perfectly clear transparent solution. Upon stimulation with methacholine chloride, the calcium and nitrogen content was considerably higher than

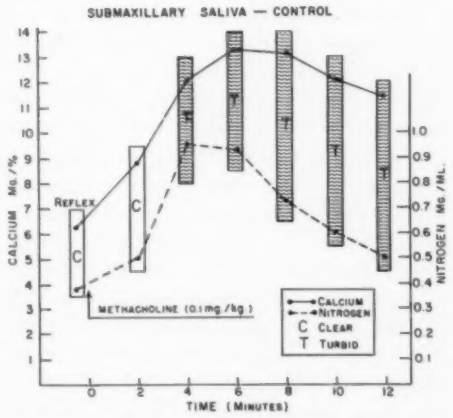

Fig. 3. Correlation of submaxillary saliva appearance and calcium and nitrogen concentrations under parasympathomimetic stimulation in a control child.

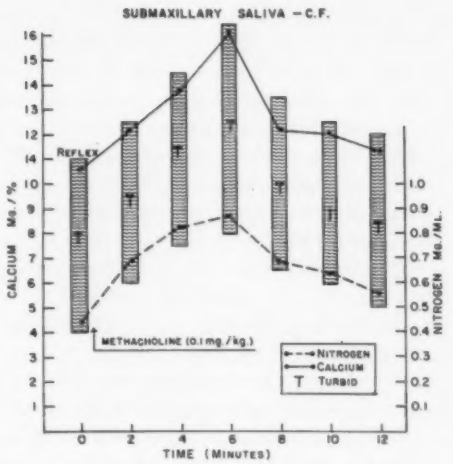

Fig. 4. Correlation of submaxillary saliva appearance and calcium and nitrogen concentrations under parasympathomimetic stimulation in a child with cystic fibrosis.

in the adult. As the peak of response was approached, the saliva appeared turbid and continued so throughout this experiment in which the calcium concentration did not fall rapidly. A second pattern was seen in other children of the control group where turbidity appeared only at the maximum point of stimulation after which the calcium content fell rapidly, and the saliva became clear again.

Fig. 4 is representative of the calcium and nitrogen curves in children with cystic fibrosis. All such children had turbid saliva throughout the entire experiment with a high initial calcium concentration and a substantial rise in both calcium and nitrogen content. The cloudy solution observed in the cystic fibrosis experiments appeared much greater than in the control group.

DISCUSSION

Clinical studies by this research group[4] have recently shown a high incidence of submaxillary gland enlargement (over 90 per cent) in patients with cystic fibrosis. The pathologic involvement associated with submaxillary gland enlargement has been described previously by Bodian.[5] Histologic

sections of this gland reveal dilated mucous acini and ductules filled with inspissated secretions. Studies have suggested that the extent of pathologic change associated with the salivary glands was correlated with the number of mucus-secreting units. Thus, the sublingual gland, composed almost entirely of mucus-secreting cells, was markedly affected while the parotid gland, containing serum-secreting cells, showed little pathologic change. The submaxillary gland, with both secretory elements, characteristically showed varying degrees of change.

The submaxillary appliance used here collects saliva from the orifices of Wharton's ducts. With various dye techniques and with particular attention being focused on the proper adjustment of the appliance, assurance was given that the saliva collected was from the submaxillary glands. In the normal individual there may be a few small interconnecting ductules from the sublingual gland which enter Wharton's duct; however, the amount of sublingual saliva secreted is less than 3 per cent of the submaxillary gland secretion. Therefore, the total amount of sublingual saliva which might be secreted into the duct would constitute only a minute fraction of the submaxillary saliva. Another factor which must be emphasized is that, in the child with cystic fibrosis, the sublingual gland may be almost completely obstructed with mucoid material and, thus, there would be little, if any, contamination of the submaxillary saliva from these glands.

In order to stimulate submaxillary gland secretion, two types of procedures have been employed. Reflex stimulation, produced by a flavored lozenge on the tongue, is the normal type of stimulatory process. This represents the nearest approach to physiologic stimulation that can be obtained in a fairly reproducible manner. All of the factors common to such type of stimulation, including the psychological attitude of the child, are brought into play under reflex stimulation and determine the resultant secretory product. Parasympathomimetic stimulation, achieved by means of injection of methacholine chloride, represents an effort to amplify the normal glandular response to its maximum point in order to characterize more easily any differences in the secretory response of control children and children with cystic fibrosis. The mean flow of submaxillary saliva stimulated reflexly in control children is slightly lower than that reported on reflex submaxillary flow in normal adults by other investigators.[6] However, the ranges are quite comparable and indicate no substantial difference in response. The difference between the control group and the children with cystic fibrosis, on the other hand, is substantial. This is also evident under conditions of parasympathomimetic stimulation. Although there are a number of alternative explanations for these differences in flow, such findings can be most readily explained by the obstruction known to be present in varying degrees in the submaxillary glands of children with cystic fibrosis. Because the flow in any given child would depend on the extent of obstruction, it is extremely difficult to utilize flow in the submaxillary gland as a measure of physiologic secretory response. In a nonobstructed gland, such as the parotid gland, our previous work has demonstrated that flow can be utilized as a measure of physiologic activity and that children with cystic fibrosis manifested a higher mean flow rate than control children.[7]

The relationship between secretion rate and electrolyte concentration has been established for several glandular systems. A positive correlation indicates a proportional mathematical relationship between an increase in flow rate and the concentration of a particular constituent. Recently, Shannon and Prigmore,[8] in a critical examination of this problem, have shown a positive correlation of the flow rate with sodium, chloride, pH, calcium, and total protein, but no significant relationship between flow rate and potassium in parotid saliva under reflex stimulation. Statistical correlation analyses have not been made with the present data. Nevertheless, it appears that similar findings can be observed in the submaxillary gland. Consequently, the higher concentration of

sodium and chloride and the lower potassium concentration under reflex stimulation observed in the control cases appear comparable to the concentrations observed in the patients with cystic fibrosis when the differences in flow rate of the two groups are taken into account. Examination of the results of parasympathomimetic stimulation reveal a reproducible pattern of response for sodium, chloride, and potassium between the two groups with comparable maximum concentrations. Such patterns of response would seem to indicate that the salivary ductules exhibit good tubular reabsorption function of submaxillary secretion under conditions of intense stimulation.

The calcium and total nitrogen concentrations observed in children with cystic fibrosis under physiologic stimulation indicate a definite alteration of the submaxillary secretory response. The high mean calcium concentration under reflex stimulation and the greater rise under parasympathomimetic stimulation indicate quite clearly the abnormality in response exhibited by patients with cystic fibrosis. The results of this study bear a striking similarity to many aspects of the previous report from this laboratory on tracheobronchial secretions,[1] where the importance of the work of Kwart and Shashoua[9] on snail mucus was cited. They proposed a structure for snail mucus in which a mucopolysaccharide is cross-linked to a mucoprotein by the divalent calcium ion and found that snail mucus could be extracted by solutions of salt containing any type of monovalent cation. It was postulated that monovalent cations could exchange with the calcium and, hence, diminish the branched or cross-linked nature of the mucus to increase solubility. They also observed that the intrinsic viscosity of snail mucus was at a maximum in a solution of calcium chloride which they explained as a lack of hydrolysis or ion exchange of calcium resulting from the presence of excess calcium. In the work relating to tracheobronchial secretions,[1] evidence was given that the calcium ion functions as a cross-linking agent in human mucoid material as well as in snail mucus. In light of these studies and the elevation in calcium found in submaxillary saliva, it is not inconsistent to envision an important role for calcium with respect to the physical properties of secretions in cystic fibrosis.

The constant presence of a turbid solution in the submaxillary saliva of patients with cystic fibrosis under physiologic reflex stimulation represents one of the most significant differences between the two groups. This continued throughout the process of secretion and, therefore, did not appear to be a washout product. During the secretory period, delicate fibrils would pass down the collection tubing. These would disintegrate on contact with the collection tube to form the turbid suspension.

The results described in Figs. 2, 3, and 4 appear to indicate that turbidity is correlated with a certain ratio existing between the calcium concentration and some organic component. In the child with cystic fibrosis, where a high calcium and total nitrogen content is secreted under physiologic stimulation, insolubility results; whereas only when the calcium and total nitrogen content are maximum at the peak of pharmacologic stimulation does this occur in the control children. This alteration continues as long as the high calcium concentration is present. In instances where the calcium fell rapidly during the descending phase of parasympathomimetic stimulation, turbidity diminished and the saliva again appeared clear.

It is not illogical to conclude that precipitation of insoluble material could, over a period of time, result in widespread obstruction of the salivary ductules. It is important to emphasize that this phenomenon occurs constantly and consistently under conditions of reflex physiologic stimulation in the child with cystic fibrosis and only in the control child when maximum stimulation with methacholine chloride is utilized. The demonstration of this material in control children subjected to intense stimulation suggests that the alteration may not be entirely dependent on the secretion of a genetically determined abnormal mucoprotein or muco-

polysaccharide. Further studies will be necessary to determine the nature of this insoluble material in order to state whether such material in the child with cystic fibrosis represents an abnormal secretory constituent or a normal constituent of salivary secretion in an altered physicochemical form.

SUMMARY

A method has been described for the construction of an appliance to be used in the collection of submaxillary saliva from children with cystic fibrosis and from control children. The data accumulated by reflex stimulation indicate a slightly lower flow rate in children with cystic fibrosis as compared with that of the controls. The sodium, chloride, and potassium concentrations are comparable when analyzed on the basis of flow. Hydrogen ion concentrations were found to be similar in both groups. The children with cystic fibrosis showed a significant elevation in calcium and total nitrogen content.

A major difference associated with submaxillary saliva was the presence of a turbid secretion from patients with cystic fibrosis as compared with a clear transparent secretion from control children under conditions of reflex stimulation. It is possible that precipitation of the insoluble material observed in these secretions could, over a period of time, result in the widespread obstruction of the salivary ductules as seen in histologic sections of the glands.

Comparable results, as described under reflex stimulation, were obtained under conditions of parasympathomimetic stimulation for all constituents studied.

The results suggest that there may be an important relationship existing between the elevated calcium and nitrogen content and the insolubility phenomenon which has been seen in all children with cystic fibrosis under physiologic stimulation.

We wish to express our appreciation to Edward Beadle, D.D.S., for his large contribution to the many technical facets of this work. Appreciation is also extended to Raymond Werther, D.D.S., for allowing the use of The Children's Hospital dental facilities and his advice on this project.

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The persistence in the human circulation of horse and human tetanus antitoxins

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FOR MANY years, large amounts of antitetanus hyperimmune horse globulin have been used in the United States, and this practice is increasing. Horse serum products are dangerous because many severe reactions and a small number of deaths occur yearly in those who receive their first injection of such products. In addition, because of possible sensitization from the first dose, a second administration of horse globulin is an even more hazardous procedure.

The use of human tetanus antitoxin would virtually eliminate foreign protein reactions, and any number of injections could be given. Furthermore, unit for unit, the ratio of effectiveness of human antibody should be far superior to antibody derived from a heterologous species since the human product should be treated by the human body as a normal constituent, whereas the heterologous horse product, as a foreign protein, would be eliminated far more rapidly by the human system.

The present study was concerned primarily with the comparison of the persistence of heterologous and homologous antibodies following passive immunization in human beings. The results also provided information on the half life of both antibodies. This was of particular interest since these antibodies contained a "natural" tag instead of the usual labeling with a radioactive tag which may alter the protein. A further advantage was that the recipients in this study were normal individuals rather than patients with hypogammaglobulinemia who have been used in other studies in accumulating similar information.[1]

MATERIALS AND METHODS

Antitoxins. The horse-globulin antitoxin was a standard commercial product (Lederle). The human antitoxin was prepared in these laboratories by the cold ethanol fractionation of pooled plasmas[2] ob-

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Dr. Stokes was unaware of the publication of this issue of the JOURNAL OF PEDIATRICS *in his honor or that the senior author had submitted this paper as a contribution.*

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Table I (Group I). The persistence of horse-globulin antitoxin in human beings (units per milliliter of serum)

	Time after antitoxin injection									
			Weeks							
Donor	*1 hour*	*3 days*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Da	-	.32	.32	.08	.04	.02	.01	.005	<.0025	<.0025	<.0025
Fr	.01	.32	.16	.08	.04	.02	.01	.0025	<.0025	<.0025	<.0025
Go	.02	.32	.32	.16	-	.02	.01	.005	<.0025	<.0025	<.0025
Hu	.02	.32	.32	.08	.04	.02	.005	.0025	<.0025	<.0025	-
La	.04	.64	.32	.08	.04	.02	.005	<.0025	<.0025	<.0025	-
Di	.01	.64	.32	.08	.04	.02	-	<.0025	<.0025	<.0025	<.0025
Mi	.02	.32	.32	.16	.04	.02	.01	.0025	<.0025	<.0025	<.0025
Na	.01	.32	.16	.08	.04	.02	.01	.0025	<.0025	<.0025	<.0025
Po	.005	.32	.32	.08	.04	.02	.01	.005	.0025	.0025	<.0025
Pr	.01	.32	.32	.08	.04	.02	.01	.005	.0025	<.0025	<.0025
Ge	.08	.64	.32	.08	.04	.02	-	.005	.0025	<.0025	<.0025

Preimmunization titers, <.0025 unit per milliliter.

tained by the repeated plasmapheresis of male donors immunized with tetanus toxoid. The plasmapheresis process has been described previously.[3] The details of the immunization procedure will be published as one of a series of papers on human plasmapheresis. All of the antitoxin activity was found in Fraction II, i.e., gamma globulin which was demonstrated to be homogenous as measured by free boundary electrophoresis.

Subjects. The individuals who received passive immunization were all female subjects between the ages of 16 and 46 years with antitoxin titers of < 0.0025 units per milliliter of serum. Male subjects could not be used since it was practically impossible to find a sufficient number who had not received active immunization during their service in the armed forces.

The desired amount of antitoxin was injected subcutaneously in the deltoid region. The entire contents of a 1,500 unit vial of the horse-globulin antitoxin was injected into the 11 members of Group I. The human antitoxin contained at least 500 units and less than 1,000 units per milliliter. The 6 individuals in Group II received 2 ml. of human antitoxin (16 per cent protein). The 10 persons in Group III received 0.25 ml. of human antitoxin. Samples of 10 ml. of blood were taken from the members of Groups I and III before immunization, 1 hour after immunization, at 3 days, and then at weekly intervals. In order to ascertain how rapidly the human antitoxin reached maximum levels in the circulation, samples were taken from the members of Group II before immunization, daily for 3 days, and weekly thereafter. The blood was allowed to clot and the serum was recovered after centrifugation.

Antitoxin assays. Antitoxin levels were obtained by the subcutaneous injection of white mice (18 to 22 Gm.) with appropriate dilutions of the donor's serum mixed with the desired dose of toxin. Controls with a standard antitoxin of The National Institutes of Health, as well as our own standard human antitoxin, were included. It was found that the use of one mouse per dilution yielded highly reproducible results,* and this plan was used routinely.

RESULTS

The persistence of heterologous antitoxin. Table I gives the results obtained in the individuals who received the horse globulin. The antibody levels remained relatively unchanged for one week, followed by a sharp drop during the second week. From the end of the second week the decrease was

*Suggested by Dr. S. Formal of the Walter Reed Research Institute.

fairly constant, the titer being halved each successive week. Eight of the 11 titers diminished at least 75 per cent in 2 weeks; and 4 weeks after the administration of the heterologous antibody, all of the original levels were reduced by over 90 per cent. At the 7 week period, the antibody titers had returned to their preimmunization levels. The half life of the horse globulin was then calculated to be between 7 and 14 days.

The persistence of homologous antitoxin. Tables II and III show the rate of disappearance of the human antibody. Since the method of double dilutions was used, small decreases in antitoxin titers were not discerned, and, as a result, plateaus were formed at different levels of antibody.

Table II is concerned with the higher level of the antibody and it may be seen that after 2 weeks the antibody was present in the original concentration. Five weeks after the injection of antitoxin, 50 per cent remained, and even after 14 weeks appreciable amounts of antitoxin were present, i.e., 0.01 unit per milliliter. The half life of the homologous antibody at the high level was about 4 weeks.

Table III gives the results found in members of Group III who received the smaller amount of antitoxin, and it may be seen that after 3 weeks the levels are undiminished. After 7 weeks, most of the donors maintained 50 per cent of the original level, and after 10 weeks about 25 per cent of the original antibody was found to be present.

The appearance of the antibody in the circulation. It was deemed of interest to determine how rapidly the antibody would reach optimal levels in the circulation following injection since this would be of clinical importance in certain instances. Accordingly, samples were obtained from the

Table II (Group II). The persistence of human antitoxic gamma globulin in human beings (units per milliliter of serum)

	Time after antitoxin injection															
				Weeks												
Donor	*1 day*	*2 days*	*3 days*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
Gr	.04	.08	.08	.08	.08	.04	.04	.02	.02	.02	.01	.01	.005	.005	.0025	.0025	.0025
Ha	.16	.16	.16	.16	.16	.08	.08	.08	.04	.04	.02	.02	.01	.01	.01	–	.005
Ho	.16	.16	.16	.16	.16	.08	.08	.08	.08	.04	.04	.02	.02	.02	.02	–	.01
Ro	.08	.16	.16	.16	.16	.16	.08	.08	.04	.04	.04	.02	.02	.02	.02	–	.01
Sc	.08	.16	.16	.16	.16	.08	.08	.08	.04	.04	.04	.02	.02	.02	.02	–	.01
Mi	.16	.16	.16	.16	.16	.08	.08	.08	.04	.04	.04	.02	.02	.02	.02	–	.01

Preimmunization titers, <.0025 unit per milliliter.

Table III (Group III). The persistence of human antitoxic gamma globulin in human beings (units per milliliter of serum)

	Time after antitoxin injection											
			Weeks									
Donor	*1 hour*	*3 days*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Sp	<.0025	.02	.02	.02	.01	.01	.005	.005	.005	.005		
Se	.0025	.02	.02	.02	.02	.02	.02	.01	.01	.01	.005	.005
Sm	<.0025	–	.02	.02	–	–	.01	.01	.005	.005	.005	
Sc	<.0025	.02	.02	.02	.02	.01	–	.005	.005	.005	.005	.0025
Sh	<.0025	.02	.02	.02	.02	.01	.01	.01	.01	.005	.005	.005
Ps	<.0025	.02	.02	.02	.02	.01	.01	.01	.01	.005	.005	.005
Wa	<.0025	.02	.02	.02	.02	.01	.01	.01	.01	.01	.005	.005
Wo	<.0025	.02	.02	.02	.02	.01	.01	.01	.01	.01	.005	.005
Ro	<.0025	.02	.02	.02	.02	.01	.01	.01	.01	.005	.005	.005
Ri	<.0025	.02	.02	.02	.02	.01	.01	.005	.005	.0025	.0025	.0025

Preimmunization titers, <.0025 unit per milliliter.

individuals comprising Group II (Table II) on the first, second, and third days after injection. It may be seen that excellent levels were obtained within 1 day, and maximal levels were reached in all persons within 2 days.

Samples were taken from certain individuals 1 hour after the injection. The results depicted in Tables I and III showed that, when relatively large amounts of antitoxin were administered, a small portion reached the circulation within this short time (Table I), but with small amounts of antitoxin virtually no measurable antibody was demonstrated (Table III).

DISCUSSION

As expected, on passive immunization, the homologous antibody persisted considerably longer than the heterologous antibody. The sudden drop in titer of the horse-globulin antibody between 7 and 14 days following administration may be attributed to antibodies directed against the foreign protein which is known to appear in this interval of time; the subsequent species antigen-antibody combination was rapidly eliminated. After the second week, the decrease in antitoxin was quite constant until the preimmunization level was reached. If the continued presence of antitoxin is required in treatment of tetanus for the neutralization of continued production of toxin by hidden tetanus bacilli, then the use of homologous antitoxin, which would not be destroyed as rapidly as the heterologous antitoxin, might well be crucial for recovery.

The elimination of the homologous human antitoxin contrasted sharply with that of the heterologous antitoxin since the former remained at elevated levels for a relatively long period of time. It is apparent that the host did not regard this antibody as foreign as evidenced by the slope of the elimination curve shown in Table II which became relatively flat and, even after 14 weeks, appreciable amounts of antibody were demonstrated.

The half life of the homologous antitoxin (gamma globulin) was found to be about 4 weeks. The "natural" tag obviated the possibility of any denaturation of the protein which may occur when chemical labeling is employed. The half life of other homologous material in the newborn infant has been reported as 31 days for diphtheria antitoxin,[4] and 30 to 35 days for the Rh antibody.[5]

SUMMARY

Human subjects were passively immunized with either horse-globulin tetanus antitoxin or human gamma globulin tetanus antitoxin. The homologous antitoxin persisted at higher levels and for a much longer period of time than the heterologous. The horse-globulin antitoxin decreased very sharply after the first week, and, thereafter, followed a straight-line curve to its preimmunization level.

We are indebted to Dorothy Wolfe for excellent technical assistance and to Donald Cheater for the fractionation of the human gamma globulin antitoxin.

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Red cell mass determinations in infancy and childhood, with the use of radioactive chromium

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A NUMBER of methods to determine total circulating blood volume have been investigated. Many groups of workers[1-4] have determined red cell mass with radioactive chromium, which has been generally accepted as one of the safest and most easily handled isotopes for clinical use. The technique for adults requires the removal of 30 to 50 c.c. of blood from the patient for the purpose of tagging before reinjecting a few cubic centimeters for the determination. This, together with a considerable amount of blood sampling for the purpose of counting, makes the method inapplicable to an infant with a total blood volume of 300 to 500 c.c. It is the purpose of this paper to outline procedures to modify the method to meet the needs of infants and children.

METHODS

Properties and dosage of radioactive chromium. The properties of radioactive chromium (Cr^{51}) have been well described by others.[2] It has one outstanding quality—retention of its radioactivity within the red cell, without significant loss to the plasma, for approximately 24 hours. This provides for a very accurate counting without any attempt to correct for decay.

This isotope has been approved for human use by the Isotopes Division of the Atomic Energy Commission provided that the total dosage of radioactivity does not exceed 0.1 rep. It was our policy to keep the dosage of radioactivity as low as possible, commensurate with a satisfactory counting rate. We found that the dosage of 1 μc per kilogram of body weight would give approximately 6,400 to 12,800 counts in 5 minutes. This range gave an accuracy in counting rate within 1 to 2 per cent. This dosage is so low that even 2 or 3 repeat determinations on the same patient would never bring the total radiation dosage in excess of 0.0697 rep.

The formula devised by Marinelli, Quimby, and Hine[5-7] for β-emitters and very soft x-ray emitters was used for the calculation of total radiation dosage administered.

The total dose of the heavy metal chromium for a single determination of blood volume must never exceed 14 μg per kilogram of body weight according to safety

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standards. In our series the highest metal dose was 0.5 μg per kilogram.

Principles of red blood cell mass determination. The determination was divided into four main parts: (1) withdrawal of 8 to 10 c.c. blood in heparin, 5 c.c. of which were then tagged with Cr^{51}; (2) injection of an accurately measured 1 c.c. suspension of tagged cells into the circulation; (3) blood sampling (8 c.c. for duplicate 4 c.c. determinations) 10 minutes after injection; (4) the counting procedure, consisting of preparing a standard of 1 c.c. in a 4 c.c. hematocrit tube which was compared with 1 c.c. of packed cells drawn from the patient in the same type of hematocrit tube. Both of these were counted in the well type, scintillation counter. An analyzer was used to narrow the spectrum of emissions counted to that area where Cr^{51} is located. This "window technique" enabled a lower dosage of radiation to be used. Correction for volume of packed cells was made by microhematocrit, and a total blood volume determined.

The following formula was used[2] for calculating packed red blood cells (PRBC):

Total counts in circulation = total counts injected − total counts per minute per cubic centimeter standard × dilution of standard.

Counts per cubic centimeter packed cells in sample = net count per minute per cubic centimeter of sample.

$$\text{Red cell volume in cubic centimeter} = \frac{\text{total counts in circulation}}{\text{counts per cubic centimeter packed cells in sample}}$$

$$\text{Total blood volume} = \frac{\text{red cell volume} \times 100}{\text{hematocrit (in percentage)}}$$

If the patient had previous radioactive background in the blood, net counts were obtained from counts per minute per cubic centimeter of packed cell sample minus counts per minute per cubic centimeter of background sample.

The tests were done through a cut-down of a saphenous vein; polyethylene tubing was threaded from the saphenofemoral vein junction into the inferior vena cava for easy blood sampling.

Loss of some Cr^{51} from adherence to the needle and cut-down tubing was overcome by carefully washing the tubing with 10 c.c. of normal saline following the injection of the tracer dose. In addition, the needle that connected the polyethylene tube to the syringe was changed. Future blood sampling then could be done without the risk of extraneous radioactive contamination. Several counts were made on sections of the cut-down tubing and revealed a negligible radioactivity left in the tubing following saline washing as described above.

Table I. Blood volume in vitro

Experiment	*Expected red cell volume*	*Red cell volume by R.B.C. − Cr^{51} (c.c.)*	*Difference*		*Hematocrit (%)*
			c.c.	*%*	
D	120.0	123.5	+3.5	+2.9	42.7
E	151.0	148.0	−3.0	−2.0	39.5
F	195.0	198.7	+3.7	+1.9	41.0

Experimental setup. At first, experiments were carried out on a series of 7 rabbits in order for us to become conversant with the general technique. Following this, a second group of experiments were carried out on 10 normal dogs, and a third group on 7 dogs which had been splenectomized a week before any radioactive determinations were carried out.

At this point a technique and procedure had been devised for red blood cell mass determinations in small subjects, and these were checked in vitro with a known amount of blood.

Finally, a group of 100 children were studied before, during, and after surgical procedures.

RESULTS

In vitro. When we had devised a definite technique for red cell mass determination

from experiments on rabbits and dogs, a group of in vitro experiments were carried out in which blood-bank blood was measured volumetrically and then checked by our Cr^{51} method. The range of error in this group of in vitro experiments was from 1.9 to 2.9 per cent (Table I).

This series of experiments demonstrated the constant error of the method itself, without any physiologic interference. One of the constant errors seemed to be the random emission of radiation from the isotope itself. In spite of this, the in vitro calculations showed an accuracy to within 2.9 per cent, which convinced us that the technique was accurate enough for red cell mass determinations in vivo.

Infants and children. A group of children awaiting serious surgical procedures were studied preoperatively for their red cell mass and blood volume. A second determination was done during the time of, or immediately after, the surgical procedure in a number of cases and a comparison of the loss was made with that estimated by the surgical team. Many of these patients had a postoperative red cell mass determination within 24 hours of the completion of the operation. The patients ranged in age from 1 day to 12 years, and most of them were subjected to surgical procedures in which an appreciable percentage of their blood volume was lost.

A total of 100 patients were studied by the Cr^{51} technique, and their red cell mass and blood volumes were determined. Twenty-two of these were cardiac patients with abnormal shunts and/or cyanosis that caused them to be excluded from this report because of probable compensatory red cell and blood volume changes. Eight cardiac patients were included; 4 had coarctations of the aorta, which we thought would not alter their red cell mass or their blood volume; 4 had other cardiac defects and the blood loss was estimated only at operation and compared with the Cr^{51} calculated loss. In summary, a total of 78 patients constitute this study; 74 had a complete study to determine the average packed red cell mass and blood volume in relation to age, and 4 simply had preoperative and postoperative studies so as to compare the loss estimated by the surgical team with the estimated loss with the Cr^{51} method.

Table II gives the results of our investigations in the 74 infants and children studied completely. These are classified by age groups with average figures calculated for the PRBC per kilogram and blood volume per kilogram of body weight. The significant finding is that up to 3 months of age the PRBC and blood volume per kilogram are higher than at any other age period up to 12 years. The PRBC is 10 to 15 c.c. per kilogram higher in the first 7 days of life than any period from 3 months to 12 years. The PRBC is 3 to 5 c.c. per kilogram higher between 1 week and 3 months. The blood volume is an average of 10 c.c. per kilogram higher in the first 3 months than at any time up to puberty. Both of these figures are of significance when transfusion requirements are calculated in the neonatal and young infant as compared with the older infant or child. From these facts, we have employed the figure of 75 c.c. per kilogram for infants under 3 months of age and 65 c.c. per kilogram for those over 3 months in estimating blood volume and transfusion limits in surgical patients; in practice, it has proved both reliable and valuable.

Table III portrays the comparison of blood loss (blood volume) as estimated by the four members of the operating team and as calculated by the Cr^{51} method. Adjustments were made for blood lost from sampling in the Cr^{51} method and for blood given during the operation. The figures in the table are, therefore, the net results in each given case. Estimation of blood loss by an operating team is generally thought to be inaccurate and subject to error from a number of sources, e.g., drapes, sponges, and the like. Because of this inaccuracy and its implications, particularly for the small infant, electronic methods have been devised for weighing patients and sponges. They have been helpful, but an even more precise method of getting blood volume data was necessary to aid in the clinical care of

Table II. Packed red blood cells (PRBC) and blood volume in infants and children

	Diagnosis	*Age*	*Hematocrit (%)*	*Body weight (kilogram)*	*PRBC (c.c.)*	*PRBC (c.c. per Kg.)*	*Blood volume (c.c.)*	*Blood volume (c.c. per Kg.)*	*Average*
1 day to 7 days									
B. K.	Newborn	6½ hours	44	3.2	104	32.5	236.3	73.8	
M. C.	Newborn	1 day	50	2.65	103	38.8	206	77.7	
M. F. C.	Newborn	1 day	60	3.3	141.7	42.9	236	70.2	PRBC, 37.91
B. M.	Newborn	39 hours	57	3.8	114	30.0	225	59.2	c.c. per kilo-
B. C.	Newborn		59	1.8	98	54.4	178	98.8	gram
L. R.	Sacrococcygeal teratoma	4 days	40	2.7	82.4	30.5	206	76.3	Blood volume,
B. K.	Duodenal atresia	4 days	55	2.0	55.6	27.8	156.3	75.1	77.67 c.c. per
B. D.	Imperforate anus	1 day	43.5	2.5	102	40.8	229.2	91.7	kilogram
L. A.	Anal stenosis	1 day	52	2.9	105	36.2	202	70.0	
R. A. C.	Esophageal atresia	1 day	56.6	2.3	104	45.2	193	83.9	
1 week to 3 months									
K. C.	Ileal atresia	2 weeks	46.6	2.5	99.2	39.6	212.8	84.7	
J. K.	Hemangioma, neck	10 weeks	35	5.5	97.9	17.8	326.2	59.3	PRBC, 29.46
L. B.	Pyloric stenosis	5 weeks	34	3.3	81.1	25.8	250.2	78.5	c.c. per kilo-
D. B.	Pyloric stenosis	5 weeks	28.2	3.5	92	26.3	328	97.7	gram
H. B.	Malrotation	3 weeks	47.7	4.0	144	36.0	329	82.2	
J. B.	Pyloric stenosis	2 weeks	39	2.7	73.4	27.1	188	69.2	Blood volume,
J. D.	Well baby boy	2 weeks	40	3.0	104	32.6	260	86.6	78.0 c.c. per
D. R.	Post-bronchopneumonia	1+ month	33	4.2	93.2	22.2	287	68.4	kilogram
M. G.	Pyloric stenosis	8 weeks	35	4.2	101.7	24.2	290	69	
3 months to 1 year									
J. B.	Imperforate anus	3½ months	35	5.9	118.4	20.0	338.9	57.3	
M. F.	Hemangiopericytoma	5 months	29	9.5	141.9	14.9	489.5	51.5	PRBC, 23.35
S. D.	Wilms' tumor	4½ months	35.5	7.3	219.5	30.1	548.7	75.2	c.c. per kilo-
W. P.	Lymphangioma	11 months	28	6.3	210.0	31.9	719.0	114.4	gram
J. J.	Coarctation of aorta	5 months	32	5.7	137.0	24.0	428.5	75.1	
D. T.	Vascular ring	9 months	38.7	8.3	219	26.4	565	68.1	Blood volume,
P. E.	Thyroglossal cyst	4½ months	33.3	7.0	100	14.3	341	48.7	68.73 c.c. per
M. M.	Inguinal hernia	3½ months	38	7.35	181	24.6	482	65.5	kilogram
D. D.	Phimosis	3½ months	34.5	6.5	151.7	23.3	433.4	66.7	
L. R.	Umbilical hernia	4 months	37	5.0	120	24.0	324	64.8	
1 year to 2 years									
M. H.	Obstructive emphysema	22 months	39.5	7.7	252.2	32.8	646.6	84	
K. B.	Burn, old	13 months	35.5	9.5	198.8	20.9	560	58.9	
J. R.	Neuroblastoma, kidney	13 months	36.5	9.3	188.7	20.3	517	55.6	PRBC, 24.1 c.c. per kilo-
T. S.	Omphalocele, postoperative	13 months	39	9.5	197.2	20.8	509.5	53.6	gram
J. R.	Cyst, lung	1½ year	33	11.6	276	23.8	862	74.3	Blood volume,
D. F.	Megacolon	20 months	35.2	9.4	227	24.1	644	68.5	67.7 c.c. per
S. A.	Cystic hygroma, neck	13 months	28.0	5.0	80	16	287.7	57.2	kilogram
C. R.	Tracheo-esophageal fistula, imperforate anus	14 months	39.0	6.2	206.6	33.3	529	85.3	
T. A.	Maxillary adenitis	13 months	35.0	10.0	253	25.3	723	72.3	

Table II. Cont'd

	Diagnosis	Age	Hematocrit (%)	Body weight (kilogram)	PRBC (c.c.)	PRBC (c.c. per Kg.)	Blood volume (c.c.)	Blood volume (c.c. per Kg.)	Average
2 years to 4 years									
A. B.	Umbilical hernia	2¾ years	41.0	15.0	476.9	31.8	1,158.2	77.2	
D. W.	Multiple hemangioma, leg	3½ years	38.0	14.5	416.6	28.7	1,096.3	74.7	PRBC, 22.9
K. E.	Hypophosphatasia	2 years	36.0	9.1	224.1	24.6	622.5	68.4	c.c. per kilo-
M. Y.	Pectus excavatum	2¼ years	34.0	12.7	300.1	23.6	882.4	69.4	gram
J. S.	Pectus excavatum	3 years	32.0	15.9	414.5	26.0	1,295.3	80.2	
G. B.	Megacolon	2 years	32.5	10.0	228.7	22.9	703.6	70.4	Blood volume,
R. B.	Hemolytic anemia	3½ years	36.0	14.0	310.0	22.1	861.0	61.5	62.9 c.c. per
D. H.	Common duct stenosis	3 years	41.5	14.0	372	26.6	896.0	64.0	kilogram
4 years to 6 years									
M. W.	Hypospadius	4¾ years	41.0	15.5	424.7	27.4	1,035.8	66.8	
W. B.	Urinary infection	4½ years	38.0	22.7	568.8	25.1	1,494.7	65.7	PRBC, 26.7
G. S.	Arthritis	4 years	36.0	14.6	467.6	32.3	1,298.6	82.1	c.c. per kilo-
T. C.	Urethral caruncula	4¾ years	38.5	20.0	541.2	27.5	1,390.1	69.9	gram
M. S.	Rectourethra fistula	4½ years	37.0	14.6	373.2	28.9	1,101.1	75.4	
J. Mc.	Islet cell tumor	4 years	45.5	17.0	502.5	29.5	1,155.0	67.9	Blood volume
S. M.	Pectus excavatum	4 years	37.0	14.4	351.5	23.8	950.0	64.2	69.6 c.c. per
T. G.	Colonic polyp	6 years	36.6	14.3	393.0	27.5	1,091.0	76.2	kilogram
A. C.	Colonic polyp	4 years	31.0	16.8	306.2	18.2	975.1	58.2	
6 years to 8 years									
J. P.	Appendicitis	6 years	39.0	20.2	554.2	27.4	1,331.3	65.9	
C. S.	Abdominal pain	6½ years	33.0	24.3	421.0	17.2	1,324.2	54.3	
R. D.	Chorea	7¼ years	35.0	28.0	613.1	21.9	1,751.6	62.5	PRBC, 23.3
M. T.	Foreign body, stomach	7¾ years	36.0	22.9	681.4	25.4	1,892.2	77.6	c.c. per kilo- gram
E. S.	Laparotomy	6¼ years	38.0	22.8	545.4	23.9	1,446.3	63.0	
R. A. R.	Splenectomy	7½ years	33.0	22.4	561.7	25.0	1,702.4	75.9	Blood volume,
C. H.	Exstrophy bladder	7 years	35.2	18.9	407.6	21.6	1,199.0	63.4	66.1 c.c. per
K. K.	Pheochromocytoma	6 years	33.9	15.0	366.6	23.1	1,081	67.9	kilogram
D. S.	Phimosis	7 years	38	27.0	663	24.6	1,744	64.5	
8 years to 12 years									
W. D.	Neurogenic bladder	8 years	38.0	22.4	541.6	24.2	1,504.1	67.2	
C. R.	Appendiceal abscess	9 years	42.0	30.2	873.0	28.9	2,078.5	68.8	
D. H.	Bladder neck obstruction	9 years	38.0	27.4	626.0	22.5	1,648.4	60.1	PRBC, 25.9
D. C.	Cryptorchidism	10¾ years	38.6	25.0	684.4	27.4	1,772.7	70.9	c.c. per kilo-
D. M.	Superior mesenteric syndrome	11 years	44.0	34.5	894.4	25.9	2,033.0	58.9	gram
P. A.	Coarctation	12 years	38.0	29.5	635.7	21.7	1,673.0	56.7	Blood volume,
D. D.	Coarctation	12 years	41.0	30.0	746.0	24.9	1,800.0	62.0	66.7 c.c. per
R. B.	Coarctation	10 years	37.8	31.4	741.0	23.6	1,929.0	61.4	kilogram
H. R.	Banti's syndrome	12 years	36.0	45.4	1,544.0	34.0	4,288.0	94.4	

pediatric patients. We were interested to find that the determination of blood loss by the Cr^{51} method generally showed less loss than was estimated by the four members of the surgical team or was determined by the weighed-sponge technique.

SUMMARY

The evolution of a method for determining red cell mass in infancy and childhood with the use of radioactive chromium has been outlined.

The results of utilizing this method are

Table III. Blood loss at operation

	Estimated loss (c.c.)	*Cr^{51} loss (c.c.)*
1 day to 7 days		
L. R.	35	26
B. K.	50	56
1 week to 3 months		
J. K.	35	13
L. B.	25	30
3 months to 1 year		
J. B.	35	21
M. F.	100	121
S. D.	65	68
1 year to 2 years		
M. H.	75	66
K. B.	75	74
T. S.	75	25
2 years to 4 years		
G. B.	325	256
D. H.	600	500
K. W.	85	110
M. B.	100	116
4 years to 6 years		
J. M.	125	108
S. M.	200	110
6 years to 8 years		
J. R.	30	17
K. K.	1100	1068
D. D.	100	110
8 years to 12 years		
D. B.	120	125
P. A.	750	790

recorded in the cases of 78 infants and children, most of whom underwent an operation.

The practicality and accuracy of the isotope method have been evaluated.

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Fatal disseminated osteomyelitis due to an anonymous mycobacterium

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WELL-AUTHENTICATED instances of human infection by acid-fast organisms other than *Mycobacterium tuberculosis* have appeared in the medical literature since 1948. The majority of these have been examples of chronic pulmonary infection mimicking tuberculosis in adults or of suppurative cervical lymphadenitis in children.

The case which constitutes the basis of the present report is an example of disseminated osteomyelitis in an infant; it is noteworthy not only because of its rarity but also because of its ability to mimic, both clinically and histologically, other disease entities involving primarily the reticuloendothelial system.

CASE REPORT

A 19-month-old Negro male was admitted to the Children's Hospital of Philadelphia, Dec. 17, 1958, presenting complaints of recurrent fever, anorexia, and weight loss. He had been well until 5 weeks prior to admission when his parents noted that he began to refuse solid food, limiting the intake almost solely to liquids. One week later several subcutaneous nodules appeared in the scalp. They gradually increased in size and caused the child to rub his head constantly. In addition, discrete masses were noted in the neck, submental region, and the axillae. He became increasingly restless and lethargic. He cried when the legs were manipulated and refused to walk or bear weight. During the ensuing month progressive fatigue, periorbital puffiness, and dysphagia occurred. Between the onset of illness and admission there had been a decrease of 6 pounds in weight. Upon questioning, his parents recalled that profuse night sweats had recurred frequently throughout the last 6 to 8 weeks.

The pertinent physical findings noted on initial examination were: rectal temperature 99.4° F., pulse 120 per minute, respirations 44 per minute, blood pressure 105/65, several fixed subcutaneous nodules ranging up to 1.5 cm. in diameter over the frontal, occipital, and parietal regions of the skull, bilateral cervical and inguinal lymphadenopathy, marked hepatosplenomegaly, painful subcutaneous swellings below the left knee and above the left elbow associated with limitation of extension of the respective

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Fig. 1. Posteroanterior view of the skull showing multiple areas of osteolysis surrounded by marginal zones of sclerosis. On the right side there is a large lytic area in the vertical ramus of the mandible above the angle. A layer of the periosteal new bone extends from the lytic area down the lateral margin to the angle of the mandible.

Fig. 2. Lateral view of the skull showing multiple osteolytic lesions in the bones of the vault each of which is surrounded by a marginal zone of sclerosis. The bones of the base appear unusually dense. This is most noticeable in the mastoids which show no evidence of pneumatization.

joints as well as resistance to all forms of manipulation. The child appeared chronically ill and there was marked wasting of subcutaneous tissues.

Laboratory studies performed during the week following admission revealed the following: hemoglobin 7.4 Gm. per 100 ml., red blood cell count 4.81 million per cubic millimeter, reticulocytes 3.9 per cent, hematocrit 30.4 per cent, microcytosis and ovalcytosis, negative sickle cell preparation, and white blood cell count 17,300 per cubic millimeter, 70 per cent of which were neutrophils. Platelet count was 400,000. The urine sediment contained 8 to 10 leukocytes per high-power field and occasional hyaline and granular casts. Serum proteins were 7.7 Gm. per 100 ml. with an albumin-globulin ratio of 1.7/6.0. Old tuberculin, 1:1,000, was strongly positive by the second day.

X-rays were obtained of the skull, chest, and extremities. Within the calvarium there were multiple radiolucent defects surrounded by zones of radiodensity (Figs. 1 and 2). Similar lesions were present in almost every long bone and, in addition, many were associated with curvilinear elevations of the overlying periosteum (Figs. 3 and 4). Moderate wasting of the adjacent soft tissues was also present. Most of the ribs contained similar changes. The lung fields were clear and there was no hilar adenopathy. A pathologic fracture associated with a moderate amount of callus was noted in the ramus of the right mandible (Fig. 1).

At this time, a clinical diagnosis of Letterer-Siwe's disease was entertained. A study of bone marrow aspirate, carried out on the third hospital day, disclosed only marked myeloid hyperplasia with increase in the myeloid/erythroid ratio. No changes considered pathognomonic for Letterer-Siwe's disease were found.

An open biopsy of an osteolytic focus in the proximal left ulna was performed on the fifth hospital day. The pathologic report was acute osteomyelitis. The Haversian canals were widened and filled with a myriad of polymorphonuclear leukocytes. Granuloma formation was not in evidence at this time.

Aerobic and anaerobic cultures of some of the tissue were negative and no acid-fast bacilli were seen in histologic sections after appropriate stains.

Because of the strongly positive tuberculin reaction, para-amino salicylic acid and isonicotinic acid hydrazide were administered. Gastric washings on 4 consecutive days contained 1 to 15 acid-fast bacilli on 2 occasions and for this reason streptomycin was added to the antituberculous regimen. Despite their presence in smears no organisms could be cultured during the ensuing 6 weeks.

Subsequently, the child's general condition gradually, but progressively, deteriorated. Radiologically, there was little change in the osseous lesions. During life repeated attempts to recover a pathogen from bone were unsuccessful and numerous ancillary laboratory studies were either equivocal or negative. A bone marrow culture for acid-fast organisms carried out 5 days premortem was reported as positive 10 days post mortem.

Terminally the clinical course was complicated by repeated episodes of vomiting and persistent diarrhea, apathy, lethargy, anorexia, and progressive malnutrition despite otherwise adequate supportive measures. He died shortly after a gavage feeding on Feb. 22, 1959, in the tenth week of hospitalization and the fifteenth week of illness.

Fig. 3. Anteroposterior view of both upper extremities showing numerous osteolytic lesions in the long bones of each arm and several of the phalanges. The osteolytic areas are surrounded by marginal zones of sclerosis. The shaft of each ulna has been expanded at the site of the greatest involvement. There is a remarkable symmetry of the lesions in the 2 extremities.

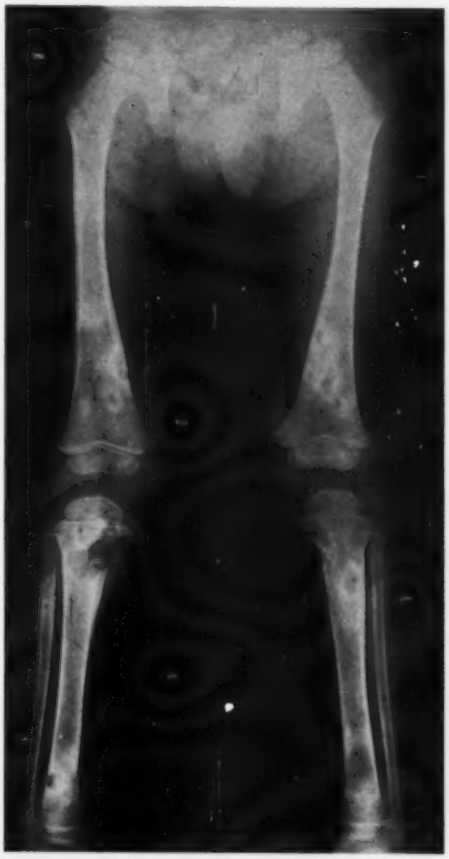

Fig. 4. Anteroposterior view of both lower extremities shows numerous osteolytic lesions in all the long bones surrounded by marginal zones of sclerosis. There is a complete destruction of the cortex on the medial side at the proximal metaphysis of the right tibia. Periosteal new bone is visible below this region along the medial shaft of the tibia. There is also periosteal new bone along both sides of the distal right femur.

AUTOPSY FINDINGS

Gross examination. The body was wasted and the abdomen distended. Cervical lymph

Fig. 5. Left humerus, longitudinally sectioned. Note thickened cortex at diaphyseal zone and punched out areas in medullary cavity.

nodes were firm and moderately enlarged. Serous surfaces of the abdominal, pleural, and pericardial cavities were translucent, moist, and smooth. Mesenteric lymph nodes were not remarkable. The external and sectioned surfaces of the pancreas were marked by irregular, soapy-white streaks in all portions. The gastrointestinal tract from esophagus to anus was unremarkable. Hepatosplenomegaly was striking, but external and sectioned surfaces of both organs revealed no distortion of anatomic pattern.

Bones taken for study included the entire left humerus, the left seventh rib, vertebrae from L_1 to L_5, the right and left petrous ridges, and 2 portions of the calvarium. Sectioned surfaces revealed similar lesions in the humerus, vertebrae, and the calvarium which were characterized by replacement of medullary tissue with numerous focal, soft, yellow-tan areas having the gross appearance of abscesses. Cortical bone, when impinged upon by the central process, was markedly thickened (Fig. 5).

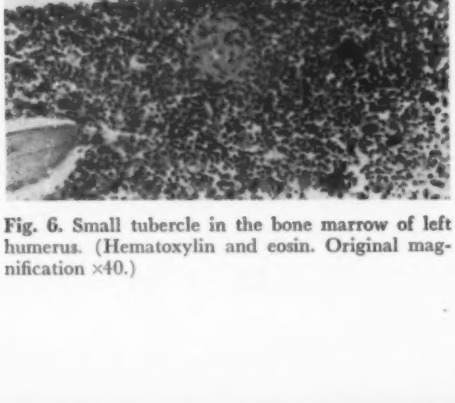

Fig. 6. Small tubercle in the bone marrow of left humerus. (Hematoxylin and eosin. Original magnification ×40.)

Microscopic examination. Bony trabeculae were absent in the necrotic foci. Hemopoietic elements were admixed with lymphocytes, plasma cells, and histiocytes, the latter often forming a tubercle-like syncytium that bore superficial resemblance to cross sections of a splenic trabeculum (Fig. 6). Histiocytic proliferation was pronounced in focal areas, particularly about sinusoidal capillaries. Except for a few eosinophilic shreds of amorphous material, the cytoplasm of each epithelioid cell was clear. Ziehl-Neelsen's stain revealed numerous intracytoplasmic acid-fast organisms (Fig. 7).

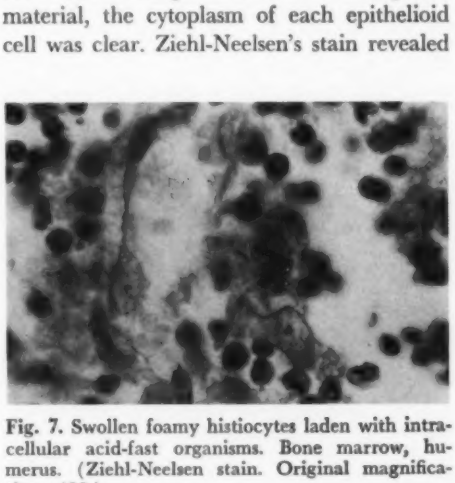

Fig. 7. Swollen foamy histiocytes laden with intracellular acid-fast organisms. Bone marrow, humerus. (Ziehl-Neelsen stain. Original magnification ×400.)

Scattered tubercles appeared throughout the liver. True giant cells of the type encountered in granulomata caused by *M. tuberculosis* were not seen.

DISCUSSION

Prior to the advent of effective antibiotic and chemotherapeutic antituberculous agents, little attention was paid to the atypical acid-fast organisms occasionally cultured from material being studied for suspected tuberculosis. They were regarded as either saprophytes or contaminants and little or no clinical import was ascribed to them. Since 1948, however, isolated reports of dis-

ease attributed to this group of organisms have been appearing in increasing number in the literature until at the present time their isolation from patients suspected of having tuberculosis poses a considerable diagnostic problem. At a recent symposium concerned with the role of these agents in human disease a classification was proposed by Runyon[1] based upon his experience and observations of hundreds of cultures recovered from clinical specimens.

In all age groups the majority of isolations were obtained from patients who exhibited clinical signs and symptoms of pulmonary disease. Cutino and McCabe,[2] however, in 1949 reported a fatal case of systemic infection by a previously undescribed organism which they designated *Nocardia intracellularis*. The patient was a child, 34 months of age, who presented complaints of anorexia, weight loss, nausea, and vomiting. Physical examination revealed an emaciated white girl with generalized lymphadenopathy and a palpable irregular upper abdominal mass. Results of laboratory studies indicated a moderate hypochromic anemia, neutrophilic leukocytosis with a marked shift to the left, hypoproteinemia and reversal of the albumin-globulin ratio, and steatorrhea. In biopsies of lymph nodes and a rectal ulcer there was a conspicuous proliferation of large foamy histiocytes resembling those seen in Gaucher's disease. In acid-fast preparations, however, large numbers of radially arranged intracytoplasmic organisms were found. Intradermal tests with old tuberculin 1:1,000, and avian tuberculin were negative. The clinical course was that of a chronically wasting disease, and she died 4½ months after the onset of the illness.

At autopsy there was generalized involvement of the reticuloendothelial system. Architecture of spleen, lymph nodes, and isolated lymph follicles were distorted by the proliferation of the previously described histiocytes. Epithelioid granulomas unassociated with necrosis were present in spleen and liver and occasional groups of multinucleated giant cells were noted in spleen and lymph nodes. Large numbers of acid-fast bacilli were present in histiocytes and epithelioid cells as well as in the multinucleated giant cells.

Another report of extrapulmonary involvement is that of van der Hoeven, Rutten, and van der Sar.[3] This was a 16-month-old male native of the Dutch Antilles who was first hospitalized because of severe anemia and diarrhea of 2 weeks' duration. On admission he was febrile (102.6° F.), dehydrated, and slightly icteric. During the first 24 hours, 6 stools containing much mucus were passed, but no enteric pathogens were recovered from the stool. Routine laboratory studies revealed a severe macrocytic anemia and mild hyperbilirubinemia (predominantly of the indirect type). The anemia responded dramatically to folic acid, and the diarrhea ceased on a regimen of sulfaguanadine and protein milk. A persistent and unexplained neutrophilic leukocytosis developed and was associated with an elevated serum protein and reversal of the A/G ratio. Readmittance 4 months later was occasioned by the appearance of generalized lymphadenopathy, slight exophthalmos, and a firm, painful swelling of the right hand. Serum phosphorus was 6.1, and the alkaline phosphatase was 34 King-Armstrong units. Roentgenograms demonstrated bilateral hilar adenopathy and a lesion of the second right metacarpal bone with periosteal and osseous reactions suggestive of syphilis or tuberculosis. Acid-fast organisms, considered saprophytes, were cultured from the gastric contents. Pirquet and Mantoux tuberculin tests, 1:1,000 dilution, were negative. No skin test with avian tuberculin was performed.

The clinical course was one of progressive bony involvement and subsequent extension of the infectious process to overlying skin with the formation of sinuses. Despite the administration of penicillin and streptomycin, there was gradual progression of lymph node enlargement and exophthalmos. Liver and spleen became palpable. Biopsy of one of the cutaneous ulcers was performed. Histologically there were numerous granulomas composed of epithelioid cells and occasional

multinucleated giant cells. In addition, numerous foam cells were scattered throughout the granulation tissue at the base of the ulcer. Acid-fast organisms were seen intracellularly similar to those seen in leprosy. Similar granulomas were observed in a needle biopsy of liver. Death occurred 16 months after onset of the illness.

At autopsy disseminated foci of osteomyelitis involving the cranium, small bones of hands and feet, long bones of extremities, and a solitary vertebra in addition to granulomatous pneumonitis were found. All lesions teemed with intracellular acid-fast organisms. Miliary granulomas were present in liver, spleen, lymph nodes, generally, and skeletal muscle, but these contained no organisms.

The similarities between this last case and that which forms the basis of the present report are most striking. Both were basically instances of fatal disseminated osteomyelitis due to an unusual acid-fast organism which proved to be resistant to antimicrobial agents usually effective against the tubercle bacillus. Of the cases cited above, the only positive tuberculin reaction was noted in the patient reported here. Dr. E. H. Runyon[1] studied subcultures of the organisms recovered from both these cases as well as from that of Cuttino and McCabe. It is his opinion that all 3 are group III, nonphotochromogenic mycobacteria, the so-called Battey bacillus. This organism, notoriously resistant to therapy, explains the poor clinical response of each patient.

Another feature of infections with these organisms, which should be stressed, is that clinically and histologically they may simulate several of the various lipodystrophies. In routine hematoxylin and eosin sections the organisms are not visible and one is impressed solely by the marked histiocytic proliferation which they elicit. In the first case described by Cuttino and McCabe, the histiocytes resembled those seen in Gaucher's disease until acid-fast preparations revealed the true nature of the lesion.[2] Caseation necrosis is not produced, but granulomas composed of epithelioid cells and occasional multinucleated giant cells are frequently observed. The presence of granulomas, however, are not pathognomonic of mycobacterial infection for the infiltrations of Letterer-Siwe's and Hand-Schüller-Christian's diseases are characteristically described as granulomatous. Indeed the clinical course and roentgenologic findings in the present case initially were suggestive of Letterer-Siwe's disease.

SUMMARY

An instance of fatal disseminated osteomyelitis in a 19-month-old Negro infant due to a nonphotochromogenic mycobacterium (Battey's bacillus) is presented together with a brief review of the pertinent literature. In addition the ability of infections due to these organisms to mimic other entities primarily involving the reticuloendothelial system is stressed.

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Airway resistance in the newborn infant

Preliminary communication

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THE frictional resistance opposing the flow of air within the bronchial tree is commonly called airway resistance.[1] Its magnitude can be expressed as the ratio of the alveolar pressure to the rate of flow during either inspiration or expiration. Thus the unit of resistance is: cm. H_2O/L./sec. The reciprocal of this: L./sec./cm. H_2O, called airway *conductance,* may be used for convenience in graphic correlations,[2] because this varies directly with lung volume. This is true not only in a single subject but also in different individuals with various sizes of lungs. According to Briscoe and DuBois[3] this relationship in healthy individuals from 4 to 87 years of age, with lung volumes ranging from 0.93 to 5.78 L., could be expressed approximately as follows: conductance = 0.24 vol. (range 0.13 vol. to 0.35 vol.). However, the same authors found differences in the regression of conductance on lung volume between children and adults.

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Apart from the need to accumulate data on this interesting section of a physiologic parameter, the particular importance of the knowledge of patency of the airways in connection with the respiratory distress syndrome of the newborn made it worth while to establish a direct method for measuring airway resistance in this age group.

METHOD

The plethysmographic method of DuBois, Botelho, and Comroe[1] for measuring airway resistance in adults has been adapted to infants. For the details of the theory, calculations and methods reference should be made to the original descriptions.[1, 3, 4] Briefly, changes in airflow are plotted electronically against the simultaneous changes in the plethysmograph pressure. The slope of this plot is a measure of airway resistance times lung volume, because plethysmograph pressure changes are proportional to alveolar pressure changes. The corresponding lung volume is measured by closing the airway and plotting, as before, mouth pressure (which is also equivalent to alveolar pressure) against plethysmograph pressure. The fact that lung volume can be derived from the ratio of these two pressures, follows from an application of Boyle's law to the volume-pressure relationship of gas

in the lungs and measurement of its volume changes in the plethysmograph.

The "baby plethysmograph" was built from a plastic incubator,* with an airtight seal around the lid and around the circular doors in its side. The infant was placed on a platform and his head kept in a face-up position by adjusting 3 foam-rubber padded braces. When the baby was asleep or reasonably quiet, he was transferred into the "box" and the lid closed (Fig. 1). A small-mesh screen pneumotachograph (flowmeter) with a small, self-inflated, rubber-cuffed face mask on its lower opening is attached to a rod which fits through an airtight hole in the top of the plethysmograph. The opposite end of the flowmeter can be closed by a mechanical shutter, operated by means of a cable release (Fig. 2). This whole assembly† is manipulated from the outside.

Fig. 1. Plethysmograph with newborn infant in position for the measurement of airway resistance and lung volume. Investigator handles flowmeter assembly and mechanical shutter from the outside. In the background switchboard and oscilloscope.

Fig. 2. Flowmeter, with face mask and mechanical shutter as seen through the door of the plethysmograph.

It was not particularly disturbing for the infant to lie with the head between the padded braces. When the mask was first lowered onto the baby's face, covering the mouth and nose, the infant either tried to avoid it or reached for it enjoying the circumoral stimulation. After a few trials most babies tolerated it well. The mask was kept on the newborn infant's face for a period of 2 to 4 breathing cycles, while the relationship of airflow to plethysmograph pressure was determined during inspiration; then the shutter was closed for at least 2 breathing cycles, and the lung volume estimated. When the shutter was closed, the infant did not hold his breath but made an immediate respiratory effort thereby making it possible to obtain an instantaneous volume measurement to which the resistance value could be related. Our subjects breathed through the nose as young infants customarily do. In some instances one could see that the infant's mouth was open, but there was no indication that he breathed through it. The results were considered to be obtained during nose breathing.

Lung compliance was measured in the supine position with the use of an esophageal catheter and balloon for detecting intrathoracic pressure changes. The results were recorded by means of the oscilloscope.

RESULTS

The results are shown in Table I. One hundred and four separate measurements of

*Supplied by Air Shields, Inc.

†Made in the machine shop of the Moore School of Electrical Engineering, University of Pennsylvania, by Robert J. Pitherington and J. Pedley.

Table I. Data on 15 newborn infants studied for airway resistance (R_A) at different lung volumes (V), some of whom were also studied for functional residual capacity (FRC) and lung compliance (C_L)

No.	*Age (days)*	*Sex*	*Weight (grams)*	*No. of observations*	*Mean volume (liters)*	*Mean R_A (cm. H_2O/L./sec.)*	*Regression coefficient ($\Delta C^*/\Delta V$)*	*Regression intercept. V axis*	*Correlation coefficient (r.)*	*Mean FRC (L.)*	*Mean C_L (L./cm. H_2O)*
1	1	M	3,200	7	0.25	11.1	0.246	0.044	0.89		
2	3	F	4,540	4	0.32	20.0	0.460	–0.0726	0.84		
3	3	M	4,550	11	0.23	22.8	0.160	0.0097	0.94	0.24	
4	¾	F	2,500	6	0.14	11.3	0.977	–0.0184	0.60	0.13	0.0052
5	1	F	2,900	12	0.16	21.9	0.246	0.00941	0.67	0.15	0.0071
6	2	M	3,600	5	0.31	14.1	–0.175	0.1309	–0.35		0.0064
7	½	M	3,540	11	0.17	13.9	0.129	0.028	0.53	0.24	0.0056
8	1	M	4,240	8	0.16	22.0	–0.005	0.0476	–0.60		
9	1	M	3,060	5	0.13	21.0	–0.009	0.0500	–0.32	0.08	0.0090
10	1	M	2,900	5	0.11	27.9	–0.056	0.0424	–0.37	0.10	0.0049
11	11	F	2,240	11	0.16	13.7	0.196	0.044	0.35	0.13	0.0056
12	17	M	1,980	5	0.13	23.9	0.144	0.0231	0.61	0.11	
13	3	M	3,290	6	0.22	16.2	0.124	0.0356	0.82	0.17	0.0032
14	1	M	3,770	4	0.22	12.9	0.511	–0.0316	0.16	0.23	0.0032
15	6	M	2,500	4	0.14	28.8	0.150	0.0175	0.63	0.16	0.0065
Total				104							
Mean of all infants	3		3,250		0.19	18.1	0.275†		0.742	0.16	0.0057
S. E.					±0.06	± 6.3			P = 0.01	±0.06	±0.0021

*C = conductance, i.e. reciprocal of airway resistance.

†Regression coefficient for the mean conductances of 15 infants on their mean lung volume (not identical with the average of the individual regression equations).

airway resistance were obtained from 15 newborn infants.* The average age of the subjects was 3 days (range ½ to 17), and their average weight was 3,250 grams (range 1,980 to 4,550). Four were premature without respiratory or other complications; 11 were born normally at full term.

The mean airway resistance of all subjects was 18.1 cm. H_2O/L./sec. (S. E. ± 6.3). Of the lung volumes measured immediately after the resistance, the mean was 0.19 L. (S. E. ± 0.06). The mean lung volume at the end of a normal expiration, i.e., the functional residual capacity, for 11 subjects was 0.16 L. (S. E. ± 0.06). The latter value gives an approximate ratio of 0.05 L. per kilogram of body weight.

The regression equation for all 104 measurements of conductance on the corresponding lung volume was: conductance in L./sec./cm. H_2O = 0.024 + 0.197 × volume in L. (correlation coefficient, r = 0.46; P = 0.001) (Fig. 3). When the average of conductances obtained on repeated measurements in each individual was plotted against the average corresponding lung volume, the regression equation was: conductance in L./sec./cm. H_2O = 0.01 + 0.275 × volume in L. (correlation coefficient, r = 0.742; P = 0.01) (Fig. 4). When the regression lines of repeated measurements for each individual were plotted, a considerable variability of the slopes became evident, but in most cases there was still a direct relationship between conductance and lung volume (Fig. 5). A surprisingly wide range of changes in lung volumes between single measurements was found, but in this series there was no obvious difference between the values or the slopes of premature and full-term babies. The average lung compliance in the 10 babies in whom it was measured was 0.0057 L./cm. H_2O (S. E. ± 0.0021).

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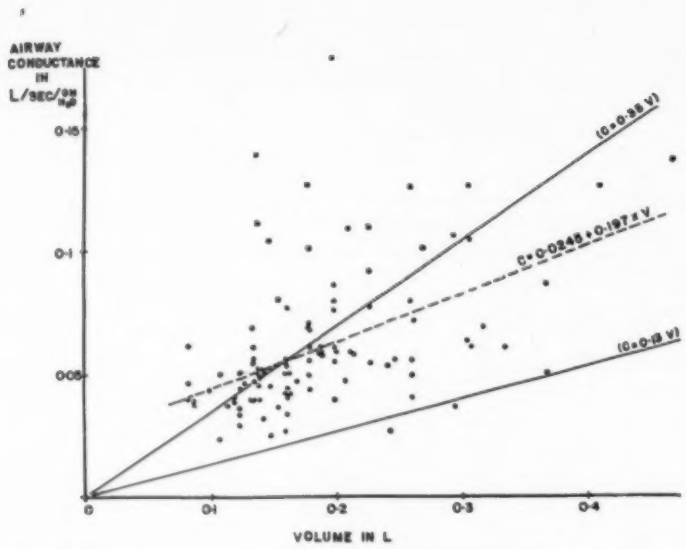

Fig. 3. Plot of 104 measurements of airway conductance (i.e., the reciprocal of airway resistance) against corresponding lung volume data from 15 newborn. Broken line: the calculated regression line for all points. Solid lines: limits of normal from the simplified prediction formula of Briscoe and DuBois.

DISCUSSION

There are no directly comparable data in the literature for the newborn. Previous reports of Cook and colleagues,[5] Swyer and Reiman,[6] and Luft, Goddard, and Roorbach[7] have given measurements of total, nonelastic lung resistance, which includes both airway and tissue resistance. These authors used a method of measuring the ratio of esophageal pressure to airflow after subtracting the elastic component from the pressure. The mean total nonelastic resistance according to these authors was 29 cm. H_2O/L./sec., 26 cm. H_2O/L./sec., and 51 cm. H_2O/L./sec., respectively. Since these values were obtained by a basically different technique which included frictional resistance of lung tissues, it is sufficient to state that the present results from direct measurement of the airway resistance (18.1 cm. H_2O/L./sec.) are partially comparable and approximately of the same order of magnitude. Unfortunately the difference in techniques does not allow us to derive the absolute value of tissue resistance by subtracting the airway resistance measured with one method from the total lung resistance determined with another one.

It is more informative to compare our results on airway resistance with similar measurements on older subjects. The basis for such a comparison is the lung volume[3] at which the airway resistance is measured. In the infants here reported this volume was measured but, in addition, the functional residual capacity (FRC) was also measured. Data from the literature provides only the latter measurement in infants to which our data can be compared. Our measurement of approximately 0.05 L. FRC per kilogram of body weight is higher than that found by Klaus, Tooley, and Weaver[8] of 0.029 L. per kilogram obtained with a similar method, and than those found by Berglund and Karlberg[9] of approximately 0.024 L. per kilogram or by Tooley and coworkers[10] of 0.022 L. per kilogram with the use of gas dilution techniques. There is no satisfactory explanation for this discrepancy.

The mean airway resistance in the present

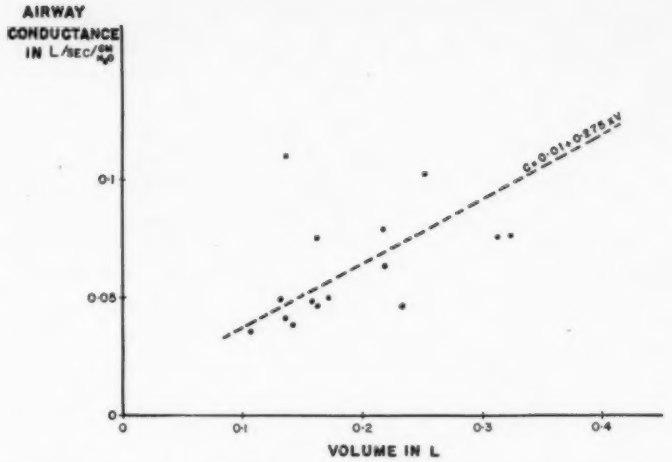

Fig. 4. The mean airway conductance for each of the 15 newborns as related to the mean lung volumes. Broken line: the calculated regression line.

series was found to be about 20 times larger than it is in adults and about 7 times larger than in older children.[3] If the conductances were plotted against the simultaneously measured lung volumes, the general range of the values was close to the approximate over-all regression of conductance = 0.24 lung volume given by Briscoe and DuBois[3] (Fig. 6). The calculated regression coefficient of these values also agreed closely with the above. However, about half of the points fell slightly above the upper limit (C = 0.35 vol.) of the simplified prediction for normal adults and children formulated by the same authors (Fig. 3). This may be due either to a disproportionately large range of error, resulting from the higher sensitivity of the recording instruments used for the newborn, or it may indicate a real increase in the range of conductance. In the latter case the difference can be explained by the relatively larger ratio of the cross

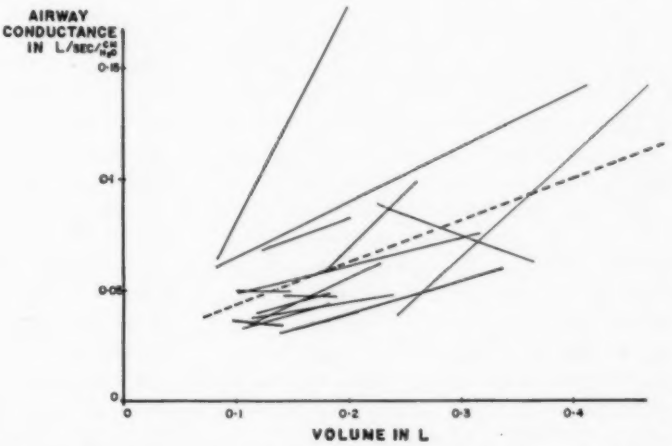

Fig. 5. Individual regression lines of 15 newborns for airway conductance on lung volume. Broken line: calculated regression line for all points.

Fig. 6. The range of values for 15 newborn infants interpolated upon the approximate relationship of airway conductance to lung volume by Briscoe and DuBois.

section area of the airways to total lung volume in newborn, as compared to older people.

The question, whether or not it is justified to extrapolate the obtained regression lines to zero conductance or zero volume, raised by Briscoe and DuBois,[3] cannot be satisfactorily answered by our data either. Although they lay, in absolute terms, very much closer to the origin, the relative distance of extrapolation to the intercept in comparison to the total volume range of the measurements was about the same in our newborn group as it was for the older ones. In fact the intercept for the newborn values was negative on the volume scale as it was for the older children, in contrast to that of adults. In individual babies replicate measurements of volume were relatively widely different (Fig. 5). It is probable that there were spontaneous changes in lung volume, but the reason for them is not known. Most of the individual regression lines show a similar correlation to the over-all regression. While the one line with the marked negative slope is obviously an artifact, there is no definite explanation for the relative steepness of at least 3 of the regression lines. They could be interpreted as manifestations of a relatively high distensibility of the airways. Since conductance has been shown to be even more directly related to the intrathoracic pressure changes than to volume changes,[11] it may be possible that in those babies with the higher regression coefficients the intrathoracic pressure changed relatively more for the same volume change than in others. Thus their airways would be pulled more widely open, resulting in higher conductance. The mean value for compliance measured as the ratio of intrathoracic pressure change to volume change in 10 of the babies agreed well with the findings of others.[5, 6] Unfortunately the lung compliance was measured in only 1 of the 3 babies who had the steep regression line for conductance on volume, and it was of average magnitude. No information was obtained on intrathoracic pressure changes measured simultaneously with the conductance.

It should be noted that in few attempts to measure the difference between nose and mouth breathing in the newborn we found that even after closing their noses with rubber stoppers or cotton plugs, the infants

chose to breathe through the remaining small leaks rather than to open their mouths. This may justify the assumption that all our results were obtained during nose breathing although the mouths of some infants seemed to be open at times. The data of Briscoe and DuBois[3] were obtained during mouth breathing. Butler[12] has shown in adults that the airway resistance increases significantly when breathing is through the nose. This relationship will have to be first established in newborn infants, before it can be decided if any correction factor is necessary for the comparison of present data with the ones of Briscoe and DuBois.

SUMMARY

1. Measurements of airway resistance at different lung volumes in newborn babies have been shown to be in general agreement with the extrapolated findings of Briscoe and DuBois in older subjects. The difference, if any, between the airway resistance during nose and during mouth breathing should be investigated in infants.

2. The derived values for conductance frequently lay above the extrapolated portion of the regression line representing the upper limit of the normal range of Briscoe and DuBois. This may be related to a larger ratio of airway cross-section area to lung volume in the newborn.

3. The hypothesis is advanced that in those newborn infants who have higher airway conductance for a given lung volume than do the majority of infants the corresponding intrathoracic pressure may have been more negative.

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The cardiac rate and rhythm in infants during induction of anesthesia with cyclopropane

Atropine versus scopolamine as preanesthetic medication

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THE use of preanesthetic medication dates from the discovery of anesthesia, when Morton's first patient was prepared with tincture of laudanum by a skeptical attending physician. Today preanesthetic medication has these goals: to allay apprehension; to decrease metabolic activity; to supplement the analgesia of the anesthetic agent; to decrease or prevent salivary secretion; and to block untoward reflexes which might occur during anesthesia. Although Waters[1] first suggested using preanesthetic medication in children, Leigh and Belton[2] gave the earliest detailed recommendations for pediatric preanesthetic medication. Leigh and Belton expressed no strong preference for either atropine or scopolamine but definitely favored the use of some parasympatholytic drug. West and Papper[3] studied 122 children from 2 to 14 years of age who had received either atropine or scopolamine for preanesthetic medication and concluded that scopolamine was superior to atropine in providing a smooth course of anesthesia. We[4] recently studied the effects of preanesthetic medication on 292 children from 6 months to 12 years of age and found that scopolamine had certain advantages in these older children. Scopolamine supplements preanesthetic sedation and causes significantly less salivary secretion during induction of anesthesia. In infants under 6 months of age the principal aim of preanesthetic medication has been the blocking of vagal cardiac reflexes during anesthesia. Infants are relatively more susceptible to these reflexes than are older individuals, and the resulting bradycardia is of graver consequence to them. Belladonna derivatives, i.e., atropine and scopolamine, have been used for this purpose. Smith[5] has preferred atropine, whereas Deming[6] has preferred scopolamine. Others have suggested a wide variety of parasympatholytic drugs, such as l-hyoscyamine (Bellafoline), oxyphenonium (Antrenyl) and methantheline (Banthine).[7-10]

In contrast to the usual pediatric dose, anesthesiologists, for preanesthetic medication, have generally administered from 4 to 20 times the minimal effective dose of atro-

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Table I. Summary of data on 48 patients

Age (months)	*Sex*	*Weight (pounds)*	*Interval time (min.)*	*Induction time (min.)*	*Pulse decrease (beats/min.)*	*Peak alveolar CO_2 (mm.Hg)*	*ARR*
Scopolamine							
2	M	10.5	35	5	60	50	0
3	M	12.0	65	6	78		+
2	F	11.2	90	4	100		0
6	M	18.9	77	5	86		+
3	M	15.0	59	3	100		+
2	M	9.1	66	4½	116		+
4	M	14.0	75	6	110	39	+
4	M	11.0	75	5½	80	43	+
3	F	12.3	50	8	30	46	+
2	M	10.1	50	10	110		+
3	F	14.6	40	4	90	39	+
4	M	13.5	75	6	70	39	+
4	M	16.0	65	3	126	43	+
2	F	12.0	60	6	20	43	0
3	M	12.2	60	6	90	49	0
3	F	12.8	45	6	100	39	+
2	M	10.8	80	9	106	33	+
3	M	7.8	35	8	54	39	+
1	M	10.8	60	6	80		+
2	M	9.0	100	4	80	43	+
4	M	15.1	46	6	110	50	+
2	M	9.0	68	6	115	36	+
4	F	11.2	120	3	132		0
Mean ± SD							
3.0 ± 1.1		12.1 ± 2.8	65 ± 20.6	5.7 ± 1.8	89 ± 28.4	42 ± 5	18
Atropine							
6	M	12.5	47	6	32	43	0
2	M	12.0	50	3¾	70		0
3	M	11.3	60	6	50		0
1	M	8.5	102	6	10		0
2	M	10.1	65	6	30		0
3	M	9.5	100	7	115		0
3	M	9.1	77	4	50		+
5	F	12.5	80	3	67		+
6	M	22.3	50	7	92		+
4	F		60	5	32	46	+
5	M	16.0	115	9	100	41	+
4	M	13.2	112	7	90	39	+
1	M	9.8	60	6	22	50	0
3	M	13.1	55	5½	90	39	+
4	M	15.0	70	6	100		+
4	M	18.0	65	4	30	50	0
1	M	7.8	70	8	52	43	0
2	M	14.3	40	5	40	39	0
1	M	7.8	65	11	32	36	0
3	M	17.0	58	6	40		0
2	M	10.8	33	6	30	43	0
3	M	12.0	45	6	30	43	0
2	M	10.5	45	5	10	39	0
4	M	15.3	55	4½	40	50	0
3	M	15.8	105	7	100	37	0
Mean ± SD							
3.1 ± 1.4		12.7 ± 3.6	67.4 ± 22.9	6.0 ± 1.7	54.2 ± 31.4	43 ± 5	8

Table II. Incidence of arrhythmias in infants during induction of anesthesia with atropine and scopolamine

	Arrhythmias	*No arrhythmias*	*Total*
Atropine	8	17	25
Scopolamine	18	5	23
Total	26	22	48

χ^2 (with Yates correction) = 10.18
$P < 0.01$

pine (0.024 mg.) recommended by Unna for an infant.[2, 11, 12] We have given seven times Unna's dose to thousands of infants without obvious side effects associated with atropine toxicity, i.e., central nervous system stimulation, tachycardia, and hyperthemia. Unna and associates measured only the minimal effective dose for blocking salivary secretions. The effective dose of parasympatholytic drugs for blocking cardiac reflexes is larger than the dose necessary for blocking salivary secretions.[13]

Atropine has been shown to be more effective in blocking cardiac reflexes in adults than is scopolamine.[14] To obtain valid data on the comparative effect of atropine and scopolamine in preventing bradycardia and arrhythmias in infants a double blind study was devised.

METHOD

Infants from under 1 month to 6 months of age about to undergo surgical operations were selected as subjects. They were given 0.15 mg. of atropine or 0.15 mg. of scopolamine intramuscularly approximately one hour before induction of anesthesia. The order of administration of the drug was randomized, and the identity of the drug was unknown to the observer and to the anesthesiologist. A protocol previously described for the study of preanesthetic medication was completed for all subjects.[8] Induction of anesthesia was begun by the use of to-and-fro cyclopropane with bag and mask, with flows of 600 c.c. per minute of cyclopropane and 800 c.c. per minute of oxygen. Ether was gradually added to the system. When Plane III of Stage III was reached, and tracheal intubation was performed, the study was terminated.

The electrocardiogram (Lead II) was recorded continuously on a Grass model 5 polygraph from the preinduction state to the end point of the experiment. The heartbeat was also monitored through a stethoscope. In patients selected at random the alveolar carbon dioxide was monitored by the Liston-Becker model L-BI infrared CO_2 analyzer and recorded on the same polygraph. Arrhythmias were noted and tabulated.

The decrease in pulse rate during the experiment was calculated by subtracting the lowest pulse rate recorded during the experiment, whether it occurred during an arrhythmia or not, from the pulse rate obtained prior to administration of the preanesthetic medication.

The effect of atropine, scopolamine, and arrhythmias, respectively, on decreases in pulse rate was determined by the method of analysis of variance.[15]

RESULTS

Forty-eight patients were studied, of whom 25 received atropine and 23 received scopol-

Table III. Types of arrhythmias occurring during induction of anesthesia in infants

Arrhythmias	*Scopolamine*	*Atropine*	*Total*
Inverted P wave	2	2	4
Nodal rhythm	14	5	19
Atrial premature ventricular contraction	2	0	2
S-T segment shift	1	0	1
Nodal premature ventricular contraction	2	0	2
Wandering pacemaker	5	5	10
Ventricular premature contraction	1	0	1
First-degree heart block	0	1	1

amine. The two groups were comparable in age, weight, sex, time interval from administration of drug to induction of anesthesia, and induction time (Table I).

Twenty-six of the 48 infants developed arrhythmias; 8 of them had received atropine, and 18 had received scopolamine. The difference in incidence of arrhythmias between the two groups is highly significant ($p < .01$) (Table II). The arrhythmias of the 8 patients given atropine were supraventricular in origin, whereas the arrhythmias of the 18 patients given scopolamine were both atrial and ventricular (Table III). All of the ventricular arrhythmias in the scopolamine group were preceded by supraventricular arrhythmias.

Table IV. Decrease in pulse rate during induction of anesthesia in beats per minute. Scopolamine is associated with an additional decrease of 23. Arrhythmias are associated with an additional decrease of 25

	Arrhythmias	*No arrhythmias*
Atropine	77.6 ± 23.7*	43.1 ± 27.5*
Scopolamine	91.2 ± 23.4*	80.4 ± 37.9

Scopolamine, $\Delta = -23$ beats per minute ($P < 0.05$).†

Arrhythmias, $\Delta = -25$ beats per minute ($P < 0.05$).†

*Mean ± SD.
†Computed by the method of Snedecor.

The mean pulse rate immediately before induction of anesthesia was 192 per minute in the atropine group and 188 in the scopolamine group. The difference is not significant. All of the patients had some decrease in pulse rate during the experiment. The smallest mean decrease in pulse rate occurred in patients who were given atropine and who had no arrhythmias. The analysis of variance of the decreases in pulse rates of the four groups of patients (atropine without arrhythmias, atropine with arrhythmias, scopolamine without arrhythmias, and scopolamine with arrhythmias) shows that the occurrence of arrhythmias as contrasted with no arrhythmias is associated with an additional average decrease in pulse rate of 25 beats per minute. Scopolamine as contrasted with atropine is associated with an additional average decrease in pulse rate of 23 beats per minute ($p < .05$) (Table IV). Patients who received scopolamine had a mean decrease in pulse rate of 89 beats per minute regardless of the occurrence of arrhythmias, and those who received atropine had a mean decrease in pulse rate of 54 beats per minute regardless of the occurrence of arrhythmias. This difference is a manifestation of: (1) the higher incidence of arrhythmias and (2) the greater decrease in pulse rates of infants who received scopolamine than of those who received atropine.

Table V. Mean interval time in minutes between administration of drug and induction of anesthesia

	Arrhythmias	*No arrhythmias*
Atropine	77.4 ± 22.9*	62.6 ± 20.9*
Scopolamine	62.9 ± 15.9*	73.0 ± 29.3*

*Mean ± SD.

In the patients given atropine, the mean interval of time before induction among the 8 patients who developed arrhythmias tended to be greater than that among those who did not develop arrhythmias (Table V). We believe that the measure of protection afforded by the atropine was probably wearing off by 75 minutes after administration of the drug.

An analysis of the weights and ages indicates that the older and heavier infants tended to be more suceptible to arrhythmias, especially in the atropine group. Therefore, the infants were divided by age into 3 groups of nearly equal numbers: 2 months

Table VI. The relation of age and arrhythmias in the patients given atropine

Age (months)	*No. of children*		
	Arrhythmias	*No arrhythmias*	*Total*
1-2	0	9	9
3	2	5	7
4-6	6	3	9

Bross's[16] $\chi^2 = 8.84$.
$P(\chi^2) < 0.01$.

and under; 3 months; and 4 months and over. There is a highly significant tendency toward an increase in the incidence of arrhythmias with age in the group which received atropine ($p < .01$) (Table VI). No analysis on the basis of weight was carried out, although there appeared to be a similar relationship between weight and arrhythmias in the atropine group.

Alveolar carbon dioxide tension was measured continuously in 30 patients selected at random; of these, 15 had received atropine and 15 had received scopolamine. The mean peak alveolar carbon dioxide tension in the atropine group was 43 ± 5 mm. Hg, and in the scopolamine group it was 42 ± 5 mm. Hg.

DISCUSSION

Arrhythmias and bradycardia are a real hazard during general anesthesia in infants. The circulation of the blood is inadequate during bradycardia, and arrhythmias may be the precursors of cardiac asystole or ventricular fibrillation. These data provide further evidence of the stronger activity of atropine than of scopolamine in blocking cardiac reflexes. Atropine appeared to become less effective as the time interval between the administration of the drug and the induction of anesthesia lengthened. On the basis of these data a supplementary dose of atropine is recommended when this time interval reaches 75 minutes.

The comparatively large doses of atropine used by pediatric anesthesiologists seem to be justified. The older and heavier infants had a tendency to develop arrhythmias. This was especially noted in those who received atropine, and is interpreted as an indication that the dose of 0.15 mg. is too low for infants from 3 to 6 months of age, and for those weighing more than 12 pounds. We now use a dose of 0.2 mg. in such infants.

The types of arrhythmias seen in this series of patients were similar to those described by Lurie and co-workers[17] during cyclopropane anesthesia associated with hypercarbia. Displacement of the pacemaker was the most common arrhythmia and the only precursor to ventricular arrhythmia. The carbon dioxide tensions measured during our to-and-fro high-flow technique were only slightly elevated for this age group. Lurie observed similar arrhythmias whether the carbon dioxide tension was normal or elevated. We do not believe that hypercarbia played a significant role in the development of the arrhythmias in our series.

The differences noted between the effects of the two drugs might not have occurred if another anesthetic agent had been used. Cyclopropane is a powerful vagal stimulating agent. Not all arrhythmias which occur during anesthesia are the result of cardiac vagal reflexes; some arrhythmias may be caused by sympathetic stimulation. Most arrhythmias in infants during anesthesia, however, are associated with bradycardia and displacement of the pacemaker, in contrast to the recorded observations in adults, in whom many arrhythmias occur during cyclopropane anesthesia independent of vagal stimulation.[17]

On the basis of these data, we recommend that comparatively large doses of atropine should be used as preanesthetic medication in infants under 6 months of age when cyclopropane is to be the anesthetic agent.

SUMMARY

1. A study of electrocardiographic abnormalities during induction of anesthesia with cyclopropane was made on 48 infants from less than 1 month to 6 months of age. Twenty-five of them received atropine as preanesthetic medication, and 23 received scopolamine; the dose of each drug was 0.15 mg.

2. Eight of the atropine-treated group and 18 of the scopolamine-treated group developed arrhythmias during induction of anesthesia. The difference is highly significant ($p < .01$).

3. All patients had a decrease in pulse rate during the induction of anesthesia. Those treated with scopolamine, as contrasted with those treated with atropine, had an additional average pulse decrease of 23 beats per minute. In the patients who

developed arrhythmias as contrasted with those with no arrhythmias an additional average pulse decrease of 25 beats per minute was found.

4. Older and heavier babies tended to be more prone to develop arrhythmias with this dose of atropine than did younger and smaller babies. The difference is highly significant ($p < .01$).

5. Protection against arrhythmias provided by atropine is usually dissipated by 75 minutes after the administration of the drug.

6. Atropine is recommended for preanesthetic medication in infants under 6 months of age when cyclopropane is to be the anesthetic agent.

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Physiologic characteristics of growth of the human brain

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RESEARCH in cerebral physiology has revealed that the brain has a number of unique circulatory and metabolic characteristics. Among these are a requirement for a continuous, relatively large supply of oxygen, an almost complete dependence on glucose as the food stuff requisite for function, and a circulation profoundly influenced and perhaps controlled by alterations in oxygen and carbon dioxide tensions. Clinical and laboratory observations have shown that the immature brain differs quantitatively from the mature organ with respect to the above and to such an extent that, considered in toto, there is indeed a qualitative difference between the two states of development. It is the purpose of this paper to outline briefly the pattern of morphologic change in the human brain, to comment on the functional changes paralleling this, and then to review in greater detail the physiologic characteristics of the maturational process. In the light of the vast literature on this subject and the all-encompassing nature of the term "physiologic" in its present-day usage, no presumption of completeness is made but, rather, attention is focused upon those aspects of the problem which bear directly upon the pathogenesis of injury to the immature brain.

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MORPHOLOGY

The relatively large size of the brain compared with the body as a whole during fetal life indicates its early developmental priority. In the second fetal month the brain accounts for 25 per cent of body weight, but this proportion decreases to 10 per cent at the time of birth. The rate of growth reaches a maximum in early postnatal life, and by 6 months the brain has doubled, and by 3 years has tripled its weight. At about 6 years, it is 95 per cent of its mature size with the remaining growth achieved in approximately equal annual increments of about 10 grams until the age of 20. There is no adolescent growth spurt.

The microscopic changes during the period of brain growth are cell division until the end of the sixth fetal month, followed by proliferation and branching of dendrites, extension of axons and collaterals, the emergence of Nissl substances, and the appearance of neurofibrils—all of these proceeding

simultaneously and being most prominent in the months just preceding and following birth.[1] Organizational control over the formation of nuclear masses and fiber tracts is poorly understood; it appears not to be inherent in the primitive neuroblast but, rather, depends upon chemical stimulation from the periphery, and is also influenced by synapse formation from other neurons. In certain circumstances such synapse formation is not only requisite to full and proper differentiation but, lacking it, atrophy and even death of the nerve cell may occur.[2] The neuron cannot exist by itself, but must be incorporated into a syncytium in order to survive and develop fully.

Although growth of cell bodies and of their processes constitutes a large proportion of the early enlargement of the brain, myelination, which begins prenatally and gains momentum after birth, accounts for an increasing proportion of subsequent cerebral growth. Electron microscopy has revealed this process to be the result of an invagination of the outer membrane of a sheath cell by the axon which then rotates on its own axis to produce a lamellar covering, the thickness of which is dependent upon the number of double membranes layered upon one another in the rotational process.[3, 4] Chemically, myelin is a complex lipoprotein which increases the conduction velocity of the axon. Although the implication of this with respect to the maturational process is not entirely clear, the correlation of the time of myelination with functional achievement of a given pathway has long been known,[5] and it seems likely that the rapid transmission of the impulse in both afferent and efferent pathways permitted by the myelin sheath provides the discreteness and specificity of neural action necessary for coordinated volitional activity. Not widely appreciated is the fact that myelination is a gradual and continuing process throughout the growing years with completion achieved only in adult life with no precise end point known. It seems likely that even after maturity there may be changes in the distribution of myelin which reflect the acquisition and loss of special motor or even intellectual skills. Osler's quip that "common sense nerve fibers are seldom medullated before forty"[6] may someday be found to have a foundation on an experimental basis.

FUNCTIONAL MATURATION

Reflex movement of the human fetus has been noted as early as the eighth gestational week and at 14 weeks spontaneous movement may begin to occur.[7] Such activity, including the more elaborate responses which characterize neonatal behavior, is largely a reflection of activity at a segmental level. The small contribution of the cerebral hemispheres until early infancy is convincingly apparent in observations of anencephalic or hydraencephalic infants in whom cortical tissue is grossly underdeveloped or absent altogether, yet who display the full range of neonatal reflexes with only minor alteration, if any, of spontaneous activity.[8-10] The fact that the defective functional development becomes obvious in early postnatal life in such infants is clear evidence of the rapid maturational imposition of control by the higher centers directly upon the behavior of the brain stem of the neonate. A characterization of subsequent functional activity of the growing infant has been a subject attracting the interest of several disciplines, and the vast literature on this subject is beyond the scope of this presentation and needs no comment except to say that the role of environmental factors has only recently gained appropriate emphasis. In this connection, it is of particular interest that deprivation of certain forms of sensory stimulation has anatomic consequences in the developing central nervous system,[11] a finding which lends support to the concept that behavioral pathology secondary to impoverishment of emotional stimulation, as it is mediated through visual, auditory, tactile, and proprioceptive pathways, has an actual structural counterpart. It is apparent that neurological development cannot be considered an autonomous biologic phenomenon except in its grossest aspects, and the extreme variability in the rate of achievement

of motor skills, speech, ideation, and intelligence from one individual to another is far more accountable to environmental factors than to inherent differences in neural capacity.

The development of the electroencephalograph has provided additional valuable information on the characterization of the maturational process. The first appearance of spontaneous electrical discharges in the human fetus occurs at 6 to 7 months[12] and points to this time as one of rapid emergence of certain enzyme systems and morphologic changes such as have been described by Flexner in a number of species.[13] In the neonatal period the electroencephalograph displays continuous, irregular asymmetric slow waves from all areas, which appear much the same in both the sleeping and waking states. However, arousal patterns may be elicited and appear as a flattening of wave forms or as diphasic slow waves followed by rapid oscillations of about 8 to 14 c.p.s.[14] While the amorphous electrical pattern of the newborn infant correlates well with the limited functional participation of the cortex in its behavioral activity, studies of direct local cortical stimulation and the evoked superficial cortical responses in newborn kittens have revealed patterns comparable to those of the mature animal. Purpura and others[15] believe that this reflects the relatively complete development of the apical dendritic system which, morphologically, is in sharp contrast with the underdeveloped basilar dendrites.

The changes which occur in the electroencephalogram in the early years of life are the gradual disappearance of a predominately slow rhythm of 2 to 4 c.p.s. followed by more rhythmic waves in the range of 4 to 7 c.p.s. At the age of 5 to 6 years, the faster activity of the occipital region begins to dominate the record which eventually evolves into waves of 8 to 12 c.p.s., the so-called alpha rhythm of the mature brain.[14] Throughout childhood, there is decreasing superimposition of waves of widely differing frequencies, giving the appearance of greater and greater uniformity and regularity of wave forms with increasing age. Although studies in identical twins have revealed the pattern for a given child to be highly characteristic for him,[16] the great variability in records from one normal child to another makes correlation with age only very gross. The "mature" record of certain 2-year-old children may more closely resemble the "immature" tracings of 6- to 7-year-olds rather than those of their own age group, and in neither may this finding be considered evidence of either mental or emotional maturity. Indeed, variability of electroencephalographic records in groups of normal children has been such that workable, well-defined criteria for normalcy suitable to all authorities do not exist in spite of the application of elaborate electronic and visual techniques of record analysis. Although this situation may be disillusioning to the clinician accustomed to the specificity and sensitivity of medicine's modern electronic tools, it must be remembered that the electroencephalograph is uniquely and dramatically helpful in the disclosure of well-defined electrical changes such as occur in certain types of seizure states.

GENERAL METABOLISM

Quantitative determinations of the rate of cerebral oxygen consumption in the adult human brain have revealed this to be among the highest of any organ of the body, the mean value being 3.5 ml. per 100 grams of brain per minute.[17] For a brain of average weight, this corresponds to a total of 50 ml. per minute, which is about one fifth of the basal oxygen requirement for the body as a whole. Studies in children have shown that, per unit weight of brain, the rate is considerably greater (5.2 ml. per 100 grams per minute), and it may be calculated that as much as 50 per cent of a 5-year-old child's basal oxygen consumption may be accounted for by the brain.[18] In infancy, the methodology for in vivo determinations is fraught with special difficulties, but limited data obtained for that age level suggest that the cerebral metabolic rate is lower

than that of a young child.[19] Knowledge of this function in the newborn infant must be inferred from in vitro studies which indicate that, at this time, it is at a level far below that of either the adult or young individual.[20, 21] In summary, on the basis of information presently available, the rate of oxygen consumption in the brain changes with age, rising sharply from a very low level at birth to a maximum in the young and falling to a somewhat lower level in the adult.[22]

The low cerebral metabolic rate of the newborn infant would seem the basis for its well-known ability to tolerate relatively severe degrees of anoxia, but Himwich and co-workers[21] have shown that this is also influenced by the ability of the immature brain to derive energy from anaerobic glycolysis. As is the case in skeletal muscle, the brain of the newborn infant is able to carry on the function for an appreciable period from anaerobic glycolysis which, while also operative in the adult brain, provides the latter with a much smaller fraction of the total energy requirement. These two factors offer the fetus even greater protection against anoxia than is present in the newborn infant, but in early postnatal life, when oxygen consumption of the cerebrum increases to a level higher than that present in the adult, tolerance to oxygen deprivation is less than that of the newborn infant or adult.[23]

In all observations, independent of age or species, the brain is the first organ to suffer irreversible damage from lack of oxygen; surviving individuals show no evidence of such change in other tissues. Resultant structural alterations in the brain are usually focal[24] because of the wide variations in the rate of blood flow and oxygen consumption in various regions.[25] In the light of the much lower rate of oxygen utilization by the cortex of the newborn infant than of the adult in vitro,[20] it is not surprising that the cortex is commonly uninvolved even in severe degrees of neonatal asphyxia.[26]

The requirement of the brain for glucose as a substrate for oxidation has been clearly demonstrated in a variety of investigations recently reviewed by Kety,[27] and there is little evidence that this requirement is not equally urgent during the period of brain growth. On the basis of the frequently observed low blood sugar levels in newborn and premature infants who are usually free of clinical evidence of shock,[28, 29] Himwich[21] has suggested that the newborn infant is more resistant to hypoglycemia than is the adult. However, it must be remembered that the reported levels are not profoundly low and that the immature brain may well react with fewer clinical manifestations to reductions in the blood glucose level than will that of older infants and children. Follow-up studies of idiopathic hypoglycemia suggest that, if anything, the young infant is more sensitive than the adult to glucose deprivation. Haworth and associates[30] reported the incidence of neurological sequelae to be 51 per cent among 35 infants who had developed symptoms before 6 months, whereas it was only 3 per cent among 23 infants who had sequelae from hypoglycemia which became manifest after 6 months of age. It remains to be determined whether this is the result of a more severe form of the disease which occurs in the young and perhaps is masked by a limited response of the immature nervous system, or whether there is a truly greater sensitivity of the young infant to glucose deprivation. In the absence of an exact knowledge of the energy requirements of the brain throughout infancy, it is difficult to offer an explanation for this possible difference in response to hypoglycemia in the two age groups, but it may be related to the demonstration in vitro by Winzler and co-workers[31] of radiocarbon incorporation into several amino acids from glucose. If this can be shown to occur in vivo also, it suggests that in addition to supplying energy for the growth and function of the brain, glucose may be in demand for amino acid and protein synthesis.

In addition to its carbohydrate need, the growing brain requires nitrogen for the formation of protein. This proceeds at a rapid rate as evidenced by the large quan-

tity of nucleic acids present during the developmental period, this being greatest during fetal life and gradually decreasing to maturity.[32] While actual rates of uptake of amino acids have not been determined in vivo, in vitro studies have shown C^{14}-labeled glycine to be incorporated into brain protein in fetal life at a rapid rate, but that this decreases with age.[33] The finding that C^{14} from labeled glucose becomes a part of a wide variety of amino acids of the brain of a 1-day-old mouse while in the adult it participates in the formation of only aspartate, glycine, and glutamate has suggested that the growing brain is not dependent on selective absorption of amino acids from the blood but is capable of forming these within the brain itself, probably from partially degraded products of other amino acids which are in equilibrium with glucose intermediates.[31]

In this connection it is of interest to note the role of thyroid hormone, recently demonstrated to play an important role in the rate of protein formation.[34] Sokoloff[35] found that amino acids were incorporated into brain protein of freshly weaned rats several fold more rapidly than into that of adult rats, and that thyroxin further increased this rate in the young but was without effect in the mature animal. This observation is congruous with morphologic studies which indicate the curtailment of growth of protein-containing structures of the brain of the rat, such as cell bodies, axons, dendrites, and myelin sheaths in thyroid-deficient animals.[36] While such changes proved reversible in the rat, irreversible defective cerebral development ensues in human infants unless therapy is given very early, and even then it may fail.[37] The reversibility of neuronal changes in the rat not seen in the human being may be glibly stated as due to the greater complexity of human cerebral development, and perhaps a greater interdependency of neuronal growth on synapse formation, but knowledge on this point is too fragmentary for more than speculation.

The active amino acid metabolism of the growing brain augments the importance of another trace metabolite, pyridoxine, a necessary coenzyme in a wide variety of reactions involving both fatty and amino acids. It is of particular importance in the brain in the decarboxylation of glutamic acid to γ-aminobutyric acid. This substance, the concentration of which increases sharply in the brain after birth,[38] has an inhibitory effect on neuronal excitability and is thought to play a major role in regulating or modifying neural function.[39] While dietary deficiencies of pyridoxine in adults result in only minor symptoms referable to the nervous system,[40] severe convulsive states which are resistant to the usual anticonvulsant agents appear in infants.[41] The basis for the convulsive activity would appear to be the depletion of the inhibitor, γ-aminobutyric acid, although there is some evidence that the latter's anticonvulsant action may not be due to a direct effect, but rather to its availability as a substrate for oxidative metabolism.[42]

Additional evidence of the importance of amino acid metabolism in cerebral maturation is found in the observations of individuals with phenylketonuria. In this condition, there is a failure in the enzymatic conversion of phenylalanine to tyrosine with a resulting accumulation of high levels of the former in the blood and tissues. Because tyrosine is readily available in diets and can be absorbed directly, the disorder is not a deficiency syndrome, but rather a toxic state associated with the large accumulation of phenylalanine which, by an unknown manner, acts to impair myelin formation.[43, 44] Dietary control of phenylalanine levels in children suffering from this condition attenuates seizures and reduces the severity of mental defect.[45] When such treatment is instituted in the neonatal period, the defective mental state has been completely prevented in several reported instances.[46] The toxic action of phenylalanine, therefore, appears to be greatest during the period of most rapid cerebral growth. Because the disorder is not progressive beyond the age of 5 to 8 years, it has been suggested that the defective state is primarily the re-

sult of impairment of myelination and that high phenylalanine levels may have no in- Actual observations have not yet been re- jurious effect beyond early childhood.[47] ported to bear out this speculation.

Knowledge of lipid metabolism of the growing brain is limited, but its importance is apparent as it also is involved in the process of myelination. Folch-Pi[48] has shown the latter to be associated with an increase in the content of cerebrosides and proteolipids and, to a much lesser extent, phosphatides and cholesterol. The findings of considerable amounts of fat in mitochondria[49] as well as in cell nuclei[50] indicates that lipids must play an active metabolic role in addition to being important to structural components. That fatty acids, like most amino acids, are formed within the brain rather than being absorbed from the blood was demonstrated by Waelsch and co-workers,[51] who noted incorporation of deuterium from body water into brain fatty acid.

In summary, the immature brain is characterized by: (1) a low over-all metabolic rate during fetal and neonatal life followed by a rapid rise in this rate in the young to a level higher than that in the mature individual; (2) a changing tolerance to lack of oxygen inversely proportional to the metabolic rate at a given age; (3) a very active protein and fat metabolism; and (4) a requirement for certain trace substances essential to the growth process, examples of which are thyroid and pyridoxine. In the light of the high energy requirement necessary to the synthesis of large molecules, it is not surprising that the brain gives up its partial dependence on anaerobic glycolysis in fetal life as a source of energy in favor of the fivefold greater caloric yield to be derived from the oxidative breakdown of glucose. With the completion of growth, the energy requirements of the brain are primarily for the purpose of functional activity and, therefore, a somewhat lower cerebral metabolic rate is present in the adult than during the period of rapid growth in the postnatal period.

THE CEREBRAL CIRCULATION

Determinations of total cerebral blood flow have shown that the adult brain, which represents only 2 per cent of total body weight, receives between 15 and 20 per cent of the total cardiac output.[17] In the child between 3 and 5 years of age, the proportion of cardiac output going to the brain is even higher, probably between 40 and 50 per cent; this represents not only a higher blood flow per unit weight (105 c.c. per 100 grams per minute) but also the larger proportion of the brain to body size. Values for cerebral blood flow in infancy have been found to be lower than those reported for young children.[19] In the newborn infant determinations in vivo have not been possible but the large arteriovenous oxygen difference[52] and the high blood viscosity at this time strongly suggest that it is at a very low level. Indeed some of the lowest values for cerebral blood flow observed in adults have been in instances of polycythemia vera[17] in which the high blood viscosity is comparable to that immediately after birth. It is not surprising that the birth process, as it causes increased intracranial pressure with resulting further lowering of cerebral blood flow,[53] may cause injuries to the brain associated with stasis and thrombus formation in major vascular channels.[54, 55] The curious distribution of vascular lesions which frequently involve white far more extensively than gray matter may well reflect the widely different vascularity and blood flow rates between these two divisions of neural tissue.[56]

One of the unique features of the cerebral circulation is its response to changes in tension of the respiratory gases. While increasing partial pressures of carbon dioxide result in a dilatation of cerebral vessels and an increase in blood flow, the opposite is true of oxygen.[57] A large body of experimental evidence supports the concept that this is the major homeostatic mechanism in regulating cerebral blood flow to maintain a constancy in the various regions of the brain in the face of wide variations in local metabolic activity. In view of the

similarity of the retinal circulation to the cerebral circulation with respect to embryology, physiology,[59, 60] and pathology,[61, 62] there is reason to believe that the effect of changes in oxygen tension on retinal vessels of the premature infant has implications with respect to cerebral maturation. The studies of Ashton[63, 64] on the pathogenesis of retrolental fibroplasia have shown that growing capillaries in the retina are far more sensitive than the mature vessels in showing a constrictive response to increased oxygen tension and that, in a relatively short period of time, this may result in irreversible obliteration. After this occurs, and the retina is thereby deprived of any effective circulation, there follows a chaotic, rapid overgrowth of capillaries into the vitreous. This second phase of nondirected budding and branching of new capillary channels appears to be due to anoxia resulting from the infarcted vessels, a factor known to stimulate vessel development.[65] In the light of the many studies recently reviewed by Craigie[66] showing that the anatomic distribution and density of capillary growth in the brain are intimately related to the metabolic demands of adjacent neural tissue, it seems probable that oxygen tension itself may be the controlling factor. Regions of the growing brain which have an increasing metabolic rate would produce a degree of local hypoxia which then acts to promote vascular growth to the area and thereby restores the equilibrium. It seems highly likely that in the same way mature cerebral capillaries dilate in response to hypoxia from an increase in local functional activity[24]; immature vessels not only dilate but also bud and extend when oxygen tension falls below a certain critical level. It is noteworthy in this connection that cerebral capillaries of prematurely born infants are very incompletely developed[67] and that in utero arterial oxygen tensions, judging from determinations of oxygen saturation in cord blood at the time of cesarean section, are at a level considerably lower than that achieved after delivery when respirations have been established in ambient air.[68, 69] In view of the frequency of neurological disabilities and defective mentation among prematurely born infants, it is tempting to speculate that an important factor in their pathogenesis is a disturbance in the maturational pattern of cerebral capillaries consequent to a sudden increase in oxygen tension of the brain after birth. If this were so, one might expect to find a higher incidence of neurological sequelae among infants with retrolental fibroplasia who have been exposed to relatively high concentrations of oxygen for a prolonged period. This has not been consistently reported,[70-72] however, although Gibbs and co-workers[73] found a higher percentage of such children to have electroencephalographic abnormalities than did children of the same birth weight who were free of retrolental fibroplasia. It must be remembered that, in spite of the similarities of retinal and cerebral vessels, their anatomic configurations are quite different, the former being distributed over a surface in contact with the avascular vitreous on one side and the highly vascular choroid on the other. Whereas the latter are contained within the three dimensional substance of the brain. This must certainly bear on the sequence of events taking place in the two organs even though the initial stage of vasoconstriction is the same. In the brain, perhaps the only effect of the increase in oxygen tension at birth is some degree of inhibition of cerebral capillary growth which may be great enough to limit neuronal development. A clear understanding of the role of changes in oxygen tension on cerebral maturation obviously must await further research.

A unique feature of the cerebral circulation is the very restricted passage of substances from the blood to the brain even in the presence of very high concentration gradients. Experiments over the past fifty years in which dyes injected into the blood stream showed limited access to the brain have given rise to the concept of the "blood-brain barrier," a kind of biochemical protective mechanism whereby toxins, viruses, and other nonphysiologic agents could be excluded from the extracellular fluid of the

brain. With the introduction of radioisotopes, it became possible to study the phenomenon in a more physiologic manner and the relative impermeability of cerebral vessels was found to extend to ions and electrolytes.[74, 75] Anatomic investigations of cerebral vessels offered incomplete explanations of this unique characteristic. With the introduction of electron microscopy, several authors have found evidence that, unlike other body tissues, the brain has no demonstrable extracellular space,[76-79] a finding largely supported by measurements using S^{35} as on indicator for this compartment.[80] It appears that entry of substances into the brain involves both the capillary wall and the cell membranes. This structural relationship is consistent with the view expressed in recent reviews[74, 81] that a substance entering the brain from the blood stream does so by an active process dependent upon the metabolic characteristics of the adjacent cells. The greater rate of passage from blood to brain of a variety of substances in the immature animal[82-87] is therefore a reflection of the more active metabolism of growing cells. It is curious, however, that the relatively insoluble molecule bilirubin, when present in increased amounts in the blood, gains access to certain neuronal structures of the brain of the premature and newborn infant. Whether this is related to its role in uncoupling oxidative phosphorylation[88] or to some other as yet unknown mechanism is yet to be determined, but it is noteworthy that the distribution and type of pathologic lesion of kernicterus is identical with those produced experimentally by anoxia.[25] However, some other factor than oxygen uptake of cells must influence its manner of transfer into cells in view of the fact that in early childhood, when oxygen requirements of the brain are much greater than in the neonatal period, bilirubin does not enter brain cells even when present in high concentrations in the blood.

In this brief review in which differences in physiology between the adult and immature brain have been described, it is clear that our knowledge falls far short of that necessary for the definition of normal ranges and for the recognition of extremes which threaten normal neural development.

Parameters for the separation of transient and benign degrees of anoxia, hypoglycemia, ad bilirubinemia from those having irreversible consequences to the brain have not been defined. We are hampered in our research, not only because the immature nervous system may show no, or at least a highly altered, response at the time of a noxious influence, but also because there are few insults which leave a hallmark indicating specific pathogenesis. Our only recourse is to make detailed observations of the functions in question in an unselected population during the growing years, with special attention paid to the perinatal period when cerebral physiology differs most sharply from that of the adult. Such individuals must be followed over a period of years until such time as it becomes possible to make a complete evaluation of neurological and mental functions. At that time correlations of deficit with previously noted extremes in physiologic functions may suggest valuable hypotheses to explain some of the causes of seizure states, mental retardation, motor disabilities, and even certain aspects of behavioral aberration.

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Epiphyseal fractures

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INFLUENCES on growth may be classified in two broad groups: (a) Those of a general character which act upon all epiphyseal cartilages simultaneously and produce symmetrical abnormalities of growth; and (b) those of a local character which alter the growth of one, or, at most, a few epiphyses and give rise to asymmetric abnormalities of growth. This paper concerns itself with the latter—specifically, the response of the individual epiphysis to trauma.

HISTORICAL

The word epiphysis appeared in English medical literature as early as 1634.[1] The first suggestion of the import of this portion of bone began when Hales,[2] in 1727, noted that bones grew in length only at their ends. This was verified by Duhamel,[3] in 1742, and John Hunter,[4] in 1760, and by numerous subsequent investigators. The microscopic anatomy of the epiphyseal plate was described by Heinrich Müller,[5] in 1858. The effects of injury to the epiphyseal plate, both experimental and clinical, were recorded in the work of Ollier,[6] Vogt,[7] and Hutchinson[8] between the years 1867 and 1894. Hass,[9] in a series of papers published in 1919, analyzed the growth disturbance produced by various types of damage to the epiphyseal plate.

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TYPES OF EPIPHYSES

There are primary and secondary centers of ossification of bone. The primary center develops the metaphysis or shaft of the long bone. The secondary center of ossification forms the epiphysis.

There are 3 types of epiphyses as described by Arey[10]: (a) the pressure epiphysis, found at the ends of bone, which transmits the weight from one bone to another; (b) the traction epiphysis, which serves as an attachment for muscles; and (c) the atavistic epiphysis, which represents a part of the skeleton that was formerly a separate bone.

The functions of the epiphyses are to serve in the formation of the joint, to develop the length of the bone, and, with few exceptions, to serve as attachments for ligaments, muscles, and tendons.

ANATOMY

The epiphysis consists of a second center of ossification and the growth plate. The plate (cartilage) and that part of the diaphysis adjacent to it, the metaphysis, together constitute what is termed the growing zone of the long bone. Between the epiphysis and the diaphyseal aspect of the cartilage,

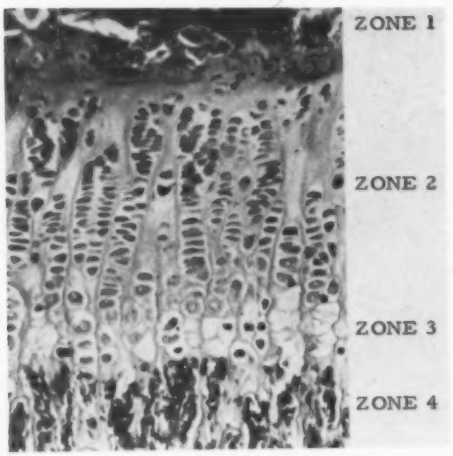


Fig. 1. Histology of the epiphyseal plate.

there are 4 different zones[11]: (1) the zone of resting cartilage, (2) the zone of proliferating young cartilage, (3) the zone of hypertrophic cartilage, and (4) the zone of provisional calcification (Fig. 1).

PATHOLOGIC ANATOMY

The weakest portion of the epiphyseal plate is the zone of hypertrophic cartilage cells at the upper limit of the zone of provisional calcification. In the studies of Hass[12] who damaged the eiphysis by trauma, of Harris[13] who produced endocrine disturbances, and of Ponseti[14] who used amino nitriles, the epiphysis always separated through the zone of hypertrophic cartilage cells, when it failed under stress.

BLOOD SUPPLY

The blood supply to a long bone is provided from three sources: the nutrient artery of the diaphysis, the epiphyseal arteries, and the periosteal arteries. The nutrient artery is evenly distributed over the central three fourths of the metaphyseal side of the growth plate. The outer fringe of the growth plate is supplied by the periosteal vessels. Both sets of vessels divide into fine arterioles, and advance in parallel toward the extended columns of hypertrophic cartilage cells. They turn back at this level in a loop with approximately one vessel oriented to each column of cartilage cells.[15] The purpose of these vessels is calcification and osteogenesis. The epiphyseal end of the bone (secondary center of ossification) is supported by blood vessels independent of the metaphyseal supply. These epiphyseal arteries penetrate the bony epiphyseal plate to gain contact with the germinal, or resting, cells of the cartilage growth plate. The purpose of these vessels is nutrient.[16, 17] With their disruption there is aseptic necrosis of the epiphysis.[13]

DIAGNOSIS

The diagnosis of fractures involving the epiphysis may be complicated, particularly in infants, by the inability to obtain an accurate history and by the lack of cooperation during examination. The roentgenogram is also difficult to interpret since the ends of the long bones are cartilaginous and the secondary centers of ossification may not be present. In older children, on the other hand, the epiphyseal plate introduces transient lines which may be interpreted by the uninitiated as fractures.[18] Again, fractures of the epiphyseal cartilaginous plate are difficult to recognize if there has been spontaneous reduction. Werenskiold[19] claims that a thin lamella of bone detached from the diaphysis and lying in the boundary between the bone and the cartilage may be the one indication of a recent separation with spontaneous reduction. Bishop[20] and most other authors feel that separation without fracture

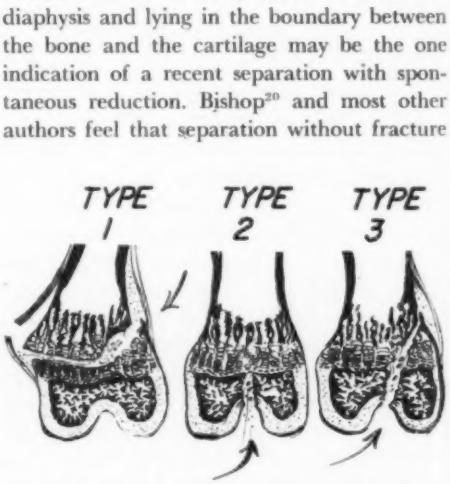
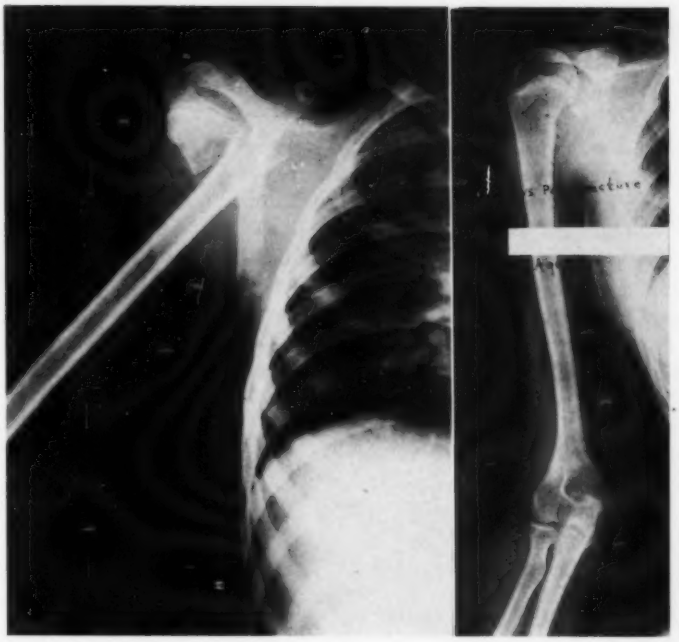


Fig. 2. Type 1, avulsion through the epiphysis; Type 2, compression with fracture of the bone and cartilaginous plate; Type 3, shearing fracture of the bone plate, cartilage plate, and metaphysis.



A *B*

Fig. 3. *A*, Type 1 fracture with displacement of proximal humerus in 10-year-old girl. *B*, Three year follow-up x-ray showing restoration to normal appearance.

(x-ray evidence of disturbance of calcified tissue) is rare. It is advisable that comparable roentgenograms be obtained of both injured and uninjured extremities in similar positions in two projections in order to find the fracture. Failure to observe this rule is frequently responsible for the error in diagnosis of a fracture in a child. Not infrequently, the injury may be overlooked for 24 hours or more, until sufficient reaction occurs to produce ecchymosis, swelling, and pain. Many of the objective signs upon which the diagnosis of a fracture are based may be absent or difficult to elicit. The so-called "epiphyseal injury without displacement" should be considered, even in the face of negative x-rays, when there are a history of trauma and persistent subjective complaints of the end of the bone. A roentgenographic diagnosis is possible in from 10 days to 3 weeks when the signs of repair make their appearance.

PRINCIPLES OF TREATMENT

The principles of treatment of fractures of the long bones of children are clear.[21-24] These principles also apply to fractures of the epiphysis, at which site they deserve their most constant application.

Alignment is the chief requirement. The fractures should not be left grossly angulated or rotated. Rotation deformities are not corrected by growth, except in infants. Therefore, when recognized, they should be corrected to avoid a permanent deformity. Angulation of less than 45 degrees is usually compatible with a perfect end result. The degree of spontaneous correctability of angular deformity is directly proportional to the number of years of growth remaining for that epiphysis.

Injury to a long bone may produce stimulation of growth.[25, 26] This is particularly true if the fractures occur in the metaphysis. This growth factor permits less concern with

overriding of the fragments which will later be compensated. In epiphyseal fractures, stimulation of growth may occur, but more frequently retardation and occasionally arrest of growth result. These changes are, for the most part, predictable.

Conservative means of treatment should be employed whenever possible. Open surgical reduction is indicated for the following epiphyseal fractures if they are not reducible by gentle manipulation: the head of the radius, the medial epicondyle, the capitellum of the humerus, the capital epiphysis of the femur, and, in children over 4 years of age, the distal epiphysis of the femur, and proximal epiphysis of the tibia.

Fig. 4*A*. Type 1 fracture involving capitellum without appreciable displacement.

Fig. 4*B*. Ten year follow-up x-ray showing nonunion and cubitus valgus deformity. There was a secondary ulnar paralysis accompanying this deformity.

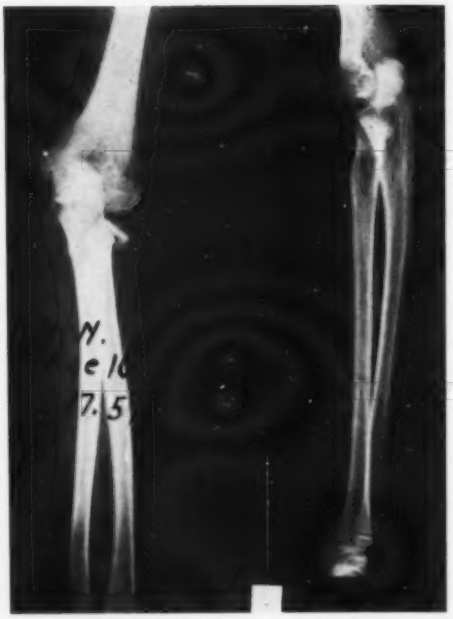


Fig. 5. Type 3 fracture showing lateral displacement of radial head in 10-year-old boy. Accurate reduction is imperative to prevent a cubitus valgus deformity.

CLASSIFICATION OF FRACTURES

Bergenfeldt[27] classified the disturbances associated with epiphyseal injuries and estimated their incidence as follows:

Group I, epiphyseal separation without any other demonstrable injury which occurs exclusively in the very young; an incidence of 7.4 per cent.

Group II, epiphyseal separation which presents signs of lamellae but without any other evidence of fracture; an incidence of 31 per cent.

Group III, epiphyseal separation combined with fracture of the diaphysis; an incidence of 50 per cent.

Group IV, epiphyseal separation combined with fracture of the epiphysis; an incidence of 6.1 per cent.

Group V, epiphyseal separation combined

with fracture of the epiphysis and the diaphysis; an incidence of 4.2 per cent.

Group VI, juxtaepiphyseal fracture; an incidence of 1.3 per cent.

Aitken[28] has recommended a simplified classification, mainly, for the purposes of prognosis, based on the mechanism of injury. Less refined, but clinically more applicable than Bergenfeldt's classification, it divides epiphyseal injuries into 3 types (Fig. 2). The following presentation is based on Aitken's classification:

Type 1, avulsion through the epiphysis with an attached periosteum or diaphyseal segment (Groups I, II, and III of Bergenfeldt).

Type 2, Compression which involves the epiphyseal bone plate with or without involvement of the cartilagenous plate (Group IV of Bergenfeldt).

Type 3, Compression fracture which involves the bone plate, the cartilage plate, and the adjacent metaphysis (Group V of Bergenfeldt).

SHOULDER

Dislocation and epiphyseal separations in the shoulder are rare in children from birth to 7 years of age. Truesdell[29] reported 3 cases in infants who were delivered by breech extraction. In the frequent adduction fracture of the humerus in the child, involvement close to the epiphyseal line may occasionally impact the fragments and crush the inner part of the epiphyseal line and thus result in late deformity.[30] Treatment, in gen-

A *B*

Fig. 6. *A,* Seven-year-old boy with Type 1 fracture of the radius. *B,* One year postfracture, modeling has been completed.

Fig. 7. X-ray taken 17 months after Type 3 fracture of distal radius of a 13-year-old boy. It shows closure of epiphysis on dorsolateral side of radius with beginning deformity.

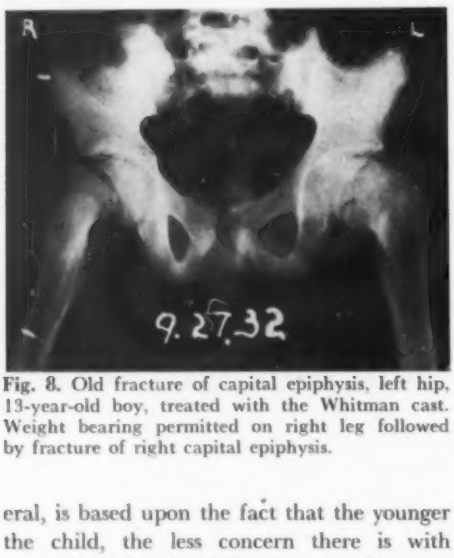


Fig. 8. Old fracture of capital epiphysis, left hip, 13-year-old boy, treated with the Whitman cast. Weight bearing permitted on right leg followed by fracture of right capital epiphysis.

eral, is based upon the fact that the younger the child, the less concern there is with anatomic reduction (Fig. 3, *A* and *B*).

ELBOW

The confluent epiphysis at the lower end of the humerus includes three main centers of ossification: one appears during the second year for the capitellum, and two appear several years later—a double center for the trochlea and one for the medial epicondyle. A fall on the flexed elbow may displace all of these epiphyses forward. The separated fragment includes the ossified center of the capitellum and the trochlea and sometimes the medial epicondyle. More frequently, as a result of a fall on the outstretched hand, one of the centers is split away from the rest. Blount[21] states that of the fractures about the elbow in children, 18.5 per cent are of the lateral condyle, and 8 per cent of the medial condyle. The lateral condyle and capitellum are fractured by a force transmitted through the radius. This can be either the Type 2 or the Type 3 fracture of Aitken. The Type 2 fracture, in which there is a separated capitellum without an adjoining fragment of metaphysis, must be openly reduced and can be accurately held by means of two crossed, fine Kirschner wires. The Type 3 fracture, with the attached fragment of the metaphysis, can frequently be replaced by manipulation. It will heal even if not accurately positioned. Neglect to reduce these fractures results in a cubitus valgus deformity and a delayed ulnar nerve palsy (Figs. 4*A* and 4*B*).

The internal epicondyle is avulsed by the common attachments of the pronator teres and the flexors of the wrist and fingers. The mechanism is usually forced abduction of the extended elbow which avulses the medial epicondyle and permits lateral dislocation and entrapment of the epicondyle within the joint. Care must be taken in reduction of the dislocation to supinate the forearm and extend the wrist and fingers and thus pull the epicondylar fragment from the joint. In order for the epicondyle to grow back, it must be accurately replaced and adequately fixed.

Fractures of the neck of the radius are reported[21] as representing 4.5 per cent of the elbow fractures. Characteristically, the buttonlike osseous center, with a thin flake of metaphysis, is broken transversely, displaced, and angulated radially (Fig. 5). The

Fig. 9*A*. Two-day-old child delivered by breech presentation, Type 1 fracture of the distal femoral epiphysis.

Fig. 9*B*. X-ray taken 25 days later, without reduction, showing bone formation along the displaced periosteum.

Fig. 9*C*. At 1 year of age there has been complete remodeling and restoration of length of the femur. (Courtesy of W. S. Armour, M.D.)

fractures are ordinarily treated by closed reduction. With residual angulations of less than 45 degrees, the results are correctible with growth. If angulation is 90 degrees, and the fragment displaced with the fracture surface against the shaft, open reduction is the treatment of choice. The radial head is never excised in a growing child, for the result will be radial deviation of the hand, shortening of the forearm, and increase in the carrying angle.

WRIST

Epiphyseal fractures of the distal end of the forearm usually occur between the ages of 6 and 10 years.[21] The radius alone, or both bones, may be involved. The deformity is that of the "silver fork" (Fig. 6, *A* and *B*). The displaced fracture is readily diagnosed on the roentgenogram. Occasionally, there is a spontaneous reduction. The diagnostic importance of a chip of the metaphysis attached to the dorsum or radial side of the epiphyseal fragment should be recognized.[32] When discovered promptly, the fracture is easily reduced. Aitken[33, 34] advocated an attempt to reduce all displacements, but stated that continued attempts or forceful manipulations were contraindicated because of the danger of irreversible damage to the epiphysis.

Early osteotomies for correction of position are unwarranted in that displacements are remodeled within a year (5 to 8 months); the loss of the volar bow is re-

Fig. 10. Medial displacement of Type 1 fracture of distal femoral epiphysis. (Despite perfect reduction of fracture fragments, x-ray films taken 2 years later showed premature closure of the femoral epiphysis.)

gained in 2 years. While there may be a temporary retardation of growth, overgrowth is the rule rather than the exception. On the other hand, fractures in this area that appear insignificant may be associated with crushing injury to the growth cells of the cartilage plate, and retarded epiphyseal growth results in the occasional case[35, 36] (Fig. 7).

Follow-up roentgenographic study is important to detect the arrest of growth. Deformity which results may be minimized by repeated osteotomies of the radius and by arrest of growth of the distal ulna.

HIP

Frank fractures of the proximal femoral epiphysis are quite rare and the prognosis is poor.[37] They are more frequently due to progressive migration of the epiphysis (adolescent coxa vara) from strain or a combination of migration and sudden trauma (Fig. 8). Roentgenograms in the anteroposterior projection will frequently show widening of the translucent epiphyseal plate. The "frog" view, or Lauenstein position, will demonstrate the femoral head and neck in profile and define the early slippage more readily than the anteroposterior view. The subjects are most frequently adolescent boys who are overweight. They generally complain of knee pain. The physician who fails to compare the motions of both hips in these children may err by simply requesting an x-ray of the knee. This x-ray will invariably show incomplete ossification of the tibial tubercle in the age group from 11 to 14 years, and may be interpreted as osteochondritis of the tibial tubercle (Osgood-Schlatter disease). The mistake of immobilizing the knee in a plaster cylinder and subsequently discovering the displaced capital epiphysis has been made too frequently. It should, however, be emphasized that not all slipped capital epiphyses occur in overweight adolescents. The active, athletic boy is also subject to strain of the capital epiphysis and even more liable to an acute fracture of the epiphysis.

In the more advanced cases, the patient walks with a limp with the foot externally rotated. The hip is adducted. A positive Trendelenburg sign is present. Physical examination demonstrates restriction in motion of the hip, particularly in abduction and internal rotation. If the slippage is severe, extension may be increased, while flex-

Fig. 11. Complete anterior displacement of tibial epiphysis in a 16-year-old boy. Injury of popliteal vessels necessitated amputation despite an anatomic position gained at reduction. (Courtesy of Charles A. Carroway, M.D.)

ion is limited. This is particularly prone to occur if the patient is placed on crutches during the management of one hip. The weight bearing extremity is then subjected to greater strain, and slip or fracture occurs.

Conservative treatment may be used temporarily, but fixation of the head on the neck by means of 3 or 4 threaded ⅛ inch pins is the method of choice even when the caput has slipped minimally. Head displacements over one-third the diameter should be reduced and then pinned. Old deformities can be corrected by osteotomy, but the incidence of avascular necrosis of the head is great.[38] The high incidence of bilateral slipping (20 to 40 per cent) must always be considered in this disease entity.

KNEE

The lower femoral epiphysis may be fractured at birth. This epiphysis usually shows an ossification center at birth[39] (Figs. 9A, 9B, and 9C). Bone remodeling will completely correct the deformity. Treatment should be by the most conservative means. According to Green and Anderson,[40] approximately 70 per cent of the growth of the femur occurs at the lower epiphysis. The epiphysis includes the whole of the articular surface of the femur and lies almost entirely within the synovial cavity of the knee .

Neer[41] stated in 1960 that 15 of 21 cases resulted in medial or posteromedial displacement (Fig. 10) of the shaft following a blow to the lateral side of the fixed extremity. He lists automobile and football injuries as the most frequent causative factors. A more recent report from The Children's Hospital of Philadelphia confirms the frequency of the same traumatic influence with fracture displacement. These injuries have been likened to the two types of supracondylar fractures of the elbow. In patients with anterior displacements, the leg is immobilized in flexion; in posterior displacement, the leg is immobilized in extension.[42] Flexion must not be forced because of the danger of compression of the popliteal vessels, thus compromising the circulation of the limb.

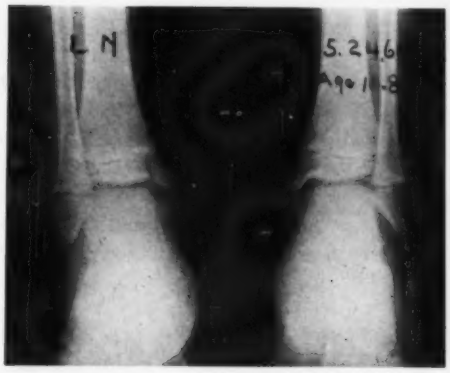


Fig. 12. Type 3 fracture of distal tibial epiphysis involving medial malleolus in a 10-year, 8-month-old boy. X-ray studies made 6 months later indicate healing without epiphyseal arrest. This patient will require prolonged observation.

Fracture separation of the proximal tibial epiphyseal cartilage is rare. It is usually the result of direct violence (Fig. 11). Aitken and Ingersoll[43] suggest that its rarity, compared with fractures of the adjacent femoral epiphysis, is due to the fact that little ligamentous attachment is found on the tibial epiphysis. Nearly all reported cases show a typical posterior displacement of the diaphysis. Pressure of the displaced shaft in the popliteal space against the tightly leashed vessels frequently produced arterial occlusion which results in gangrene and necessitates amputation. If a gentle attempt at closed reduction fails, open reduction should be undertaken at once. Since vessel injury is frequent and delay in anastomosis of the torn popliteal vessels is fraught with such a high rate of failure,[45-47] the surgeon should be prepared for the problem and take prompt action.

ANKLE

While the possibility of growth disturbance following epiphyseal injury is evident, the probability varies greatly according to the epiphysis. The ankle joint is formed by the distal fibular and distal tibial epiphyses. The medial malleolus, as part of the tibial epiphysis and because of its stress location,

is most prone to injury (Fig. 12). In the series of Carothers and Crenshaw,[48] epiphyseal injuries represented 4 per cent of 1,413 injuries of the ankle. Of 103 epiphyseal injuries in the lower extremities, 56 involved the distal tibial and fibular epiphyses. The same preponderance of injuries to the epiphyses about the ankle were reported by Bisgard and Mortenson[49] (75 per cent), Eliason and Ferguson[50] (57 per cent), and Compere[51] (69 per cent).

The Type 2 epiphyseal fracture of Aitken was the most frequently deforming one. Force, which can be exerted through bone in compression, is many times greater and probably more destructive than that which can be exerted through shearing stress.[52] In all probability, the disruption of the epiphysis in the adduction type of injury, productive of the Type 2 epiphyseal fracture, is explained by the significantly greater forces which are transmitted to the epiphysis in these injuries. The study of Carothers and Crenshaw[48] demonstrated deformity in 50 per cent of the more severe adduction injuries. Further analysis showed that the deformity was most prone to develop under the age of 12 years (75 per cent). Treatment of these deformities is by osteotomy[53] or by epiphysiodesis of the fibula, and, at times, by epiphysiodesis of the opposite ankle.

INCIDENCE AND PROGNOSIS

It is not possible to give absolute figures for epiphyseal fractures in children. Most of the reports are from institutions which receive a disproportionate number of complicated rather than average injuries. From the combined statistics of Compere,[51] Bisgard and Mortenson,[49] Eliason and Ferguson,[50] Aitken and Ingersoll,[43] Smith,[24] Lipschultz,[54] Ralston,[55] Carothers and Crenshaw,[48] and Johnson and Fahl,[56] the relative incidences of epiphyseal fractures and their complications might be estimated as follows:

In fractures of the long bones sustained by children under 16 years of age, there is a 10 to 38 per cent chance that the epiphysis will be involved, 30 to 50 per cent chance that a growth disturbance will result if the epiphysis is involved, and 2 to 10 per cent chance that the residual deformity will be significant (valgus, varus, shortening, restriction of motion, etc.). Growth may terminate entirely giving rise to shortening only, or there may be a cessation of growth in only a portion of the epiphysis. If the remainder of the epiphysis continues its normal growth activity, the end of the involved bone deviates from the normal plane producing angulation, rotation, and shortening. If growth is arrested at one end in one of two parallel bones, such as the radius and ulna or the fibula and tibia, a continued normal growth in the other bone deviates the articulating part to the short side.

SUMMARY

Although epiphyseal fractures are infrequent about the stable upper tibial epiphysis and well-protected hip, moderately rare at the shoulder, and uncommon at the distal end of the femur, they are daily problems in treatment of fractures in children at the wrist, elbow, and ankle. The prognosis in Type 2 fracture is grave. These are more frequent at the distal end of the tibia.

Age has an influence on epiphyseal fractures. In the young, separation is easy, diagnosis difficult, and repair, in the main, remarkably good. In the later years, close to the time of epiphyseal closure, the demands for manipulative reduction are greater, since spontaneous growth will be of limited use in correcting residual deformities. On the other hand, the limited growth potential will be of benefit when irreparable harm has been done to the epiphysis.

It is those children in the mid group, 8 to 12 years of age, who cause the greatest concern. With age, the bond between the epiphyseal plate and the metaphysis toughens and requires greater stress to break it, and, consequently, greater damage may be the result. If the trauma is such as to produce partial or complete cessation of epiphyseal growth, an ever-increasing, disabling deformity will occur. Because the

epiphysis is so susceptible to trauma, it cannot be too strongly stressed that it is far better to accept an imperfect reduction than to risk epiphyseal damage by a thoughtless remanipulation or operative intervention. Spontaneous realignment is dependent upon epiphyseal age rather than upon chronologic age. While caution and an expectant attitude are necessary, fractures of the epiphysis should not be viewed with an overt concern, except when x-rays demonstrate a disruption of the epiphysis itself as in Type 2 or Type 3 fractures. These fractures should be followed closely and prompt treatment instituted if and when the deformity appears. A complacent attitude should not be adopted on the basis of a satisfactory appearance of the x-ray after a few months' follow-up, since a deformity may appear as late as 2 or 3 years following injury.

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Prolonged feeding studies in premature infants

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Two hundred seventy-seven premature infants have been fed milk formulas of varied protein content during the past 3 years. These infants have been studied to determine the acceptable levels of protein intake for premature infants.

METHODS

All infants who were born at the Philadelphia General Hospital whose birth weights were less than 2,000 grams and who lived 48 hours or more are included in the study. Each infant was fed the specified formula of constant composition, beginning on the second or third day of life, depending upon weight and condition. All of the babies were given 5,000 units of vitamin A, 1,000 units of vitamin D, and 50 mg. of vitamin C daily after the fourth day. Most of the babies were discharged from the nursery when they reached 2,250 grams. At home, the infants were fed the same formula until the age of 6 months. Solid foods were introduced by the mothers at 3 months of age. Ferrous sulphate, 200 mg. per day, was given after 2 months of age. At 3 week intervals, blood urea nitrogen,[1] serum calcium,[2] phosphorus,[3] total protein,[4] and protein electrophoresis* were determined. All procedures were performed by ultramicro methods; serum from capillary blood was used. The general principles and special pipettes were described by Sanz.[5] Heights and weights were recorded semiweekly in the nursery and in the follow-up clinic. Urinary phenols were measured.[6] Food and protein efficiency ratios were calculated for the period of hospitalization. These ratios are computed with the use of the entire food intake in cubic centimeters from the time the child was at his lowest weight until he weighed 2,250 grams.

RESULTS

A. Weight. All babies in all weight groups who were fed Formulas 1 to 3 (Figs. 1 and 2) gained weight equally well, both in the hospital and after discharge. The weights

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*Spinco Procedure "B," using bromophenol blue. (Average normal value of albumin about 65 per cent.)

Table I. Composition of the five formulas

*Formula No.**	*Composition (%)*			*No. of babies*
	Protein	*Fat*	*Carbohydrate*	
1	4.1	1.5	5.8	82
2	2.7	2.8	7.8	76
3	1.5	3.5	7.0	93
4	1.1	3.5	7.0	14
5	1.1	3.5	7.0	12

*Formula 1, commercial Alacta with added Dextri-Maltose. Formula 2, commercial Lactum, standard dilution. Formula 3, commercial SMA, standard dilution. Formula 4, formula of skim milk, vegetable fat, and lactose prepared by Wyeth Laboratories. Formula 5, pooled human milk.

Formulas 1 to 3 were fed ad libitum. Formula 4 and Formula 5 (pooled human milk) were fed at the rate of 130 to 150 calories per kilogram per day.

Table II. Stool acidity for each formula

Formula No.	*Stool pH*	*No. of cases checked*
1	7.9	122
2	6.67	54
3	6.51	81
4	5.91	12
5	5.26	12

achieved are comparable to those published previously by others.[7] In the original portion of this study in which Formulas 1 and 3 were fed during a specific time interval, all infants who were on Formula 1 were later realized to be ingesting, apparently ad libitum, quantities of formula providing more than 200 calories per kilogram. Paradoxically, these infants gained poorly when compared with their matched peers on Formula 3 who drank expected amounts, which provided approximately 150 calories per kilogram. It is of special interest that the group of infants who ingested protein in excess of 8 Gm. per kilogram per day, have maintained—even after their discharge from the hospital—their original, relatively low rate of gain in weight. This raises the intriguing consideration of early "imprinting." Since the initial period when excessive amounts of Formula 1 were ingested, the infants on this formula were given approximately 150 calories per kilogram, and their rates of growth were found to be comparable with those in the other groups (Fig. 1).

Table III. Food efficiency ratio (grams per 100 c.c. formula)

Formula	*Birth weight (grams)*												*Protein efficiency* ratio (Gm./Gm.)*
	<1,000		*1,000-1,250*		*1,251-1,500*		*1,501-1,750*		*1,751-2,000*		*Average*		
	Food efficiency ratio	*No. of infants*	*Food efficiency ratio*	*No. of infants*	*Food efficiency ratio*	*No. of infants*	*Food efficiency ratio*	*No. of infants*	*Food efficiency ratio*	*No. of infants*	*Food efficiency ratio*	*No. of infants*	
1	7.3	5	5.7	5	6.3	14	6.3	28	6.8	28	6.5	80	1.6
2	6.0	2	5.7	3	6.3	5	6.8	19	6.5	27	6.5	56	2.7
3	6.3	1	7.8	5	6.4	14	6.3	28	5.9	38	6.2	86	4.1
4							5.3	14			5.3	14	4.5
5							4.9	11			4.9	11	4.5

*Weight gain in grams per gram protein intake.

Table IV. Morbidity among infants who were fed high-protein formulas*

	Formula 1		*Formula 2*		*Formula 3*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Total No. of infants	82		76		93	
Total No. followed	79	96	52	68	85	91
Diarrhea	7	8	7	14	2	2
Respiratory infections	11	13	3	6	2	2
Deaths	4	4	0	0	0	0

*Data compiled from both nursery and clinic.

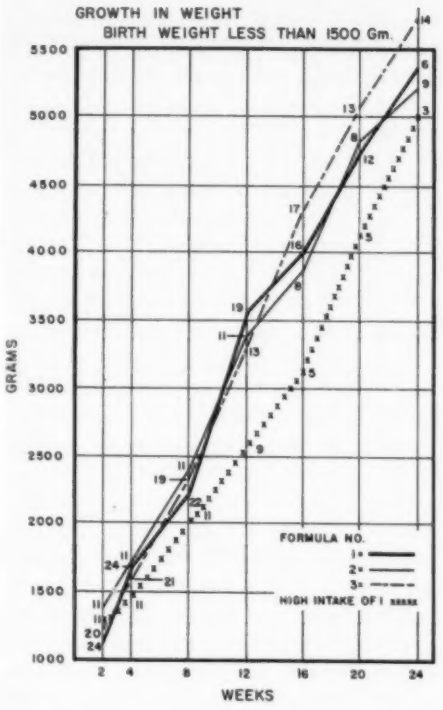

Fig. 1. Growth in weight. Birth weight less than 1,500 grams. (Small numbers on lines of all figures indicate number of babies.)

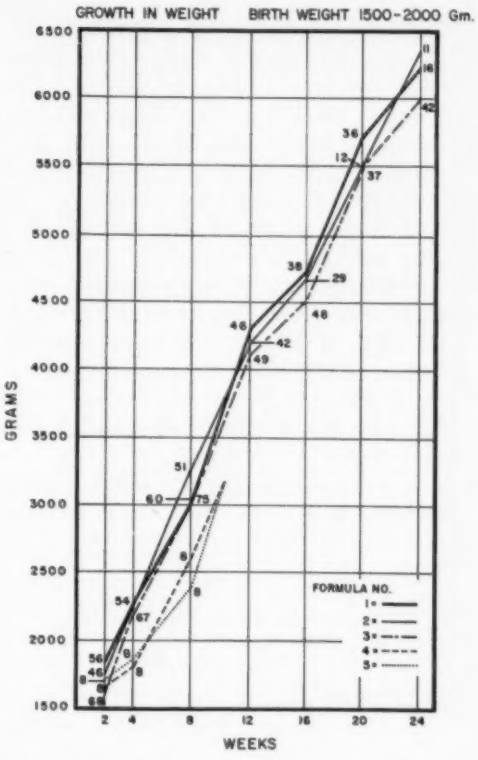

Fig. 2. Growth in weight. Birth weight 1,500 to 2,000 grams.

Babies who were fed pooled human milk (Formula 5) and Formula 4, in quantities of 130 to 150 calories per kilogram, did not grow so well as the others (Fig. 2). There was no significant difference in caloric intake between the groups receiving limited amounts of human milk or Formula 4, and between those fed Formulas 1 to 3, ad libitum, except for the small subgroup who took Formula 1 in excess of 200 calories per kilogram per day.

B. Body length. Gain in length was equivalent in all infants who were fed Formulas 1 to 3 (Fig. 3).

C. Serum urea nitrogen. Blood urea nitrogen of all babies was directly proportional to the protein content of the formula. The babies who were fed Formula 3 (1.55 per cent protein) had blood urea nitrogen levels consistently near 10 mg. per cent. Those who were fed Formula 1 (4.1 per cent protein) had elevated blood urea nitrogen, not only for the duration of the hospital stay,

Fig. 3. Growth in length. Birth weight 1,500 to 2,000 grams.

but for 4 to 6 months when they were taking a mixed diet (Figs. 4 and 5). The babies who were fed pooled human milk or a milk with its protein equivalent had a consistently low blood urea nitrogen. Birth weight, as an index of maturity, had little apparent effect on levels of the blood urea nitrogen.

D. Serum proteins. Except for the babies who were fed human milk or Formula 4 (with 1.1 per cent protein), the total serum proteins of all babies were remarkably similar (Figs. 6 and 7). Babies who were fed human milk or milk with its protein equivalent had lower total serum proteins (Fig. 7). Likewise, electrophoretic patterns of protein were similar on all formulas.

E. Stool acidity. The pH of the stools of infants who were fed each of the formulas was measured. The results are given in Table II. The higher the protein content of the formula, the higher the average pH of the stool.

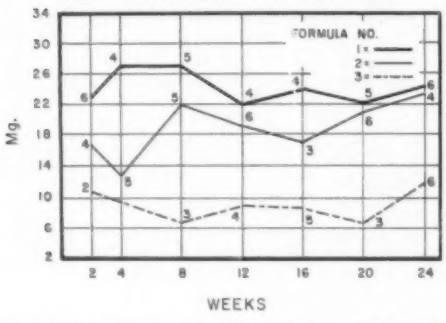

Fig. 4. Blood urea nitrogen. Birth weight 1,000 to 1,500 grams.

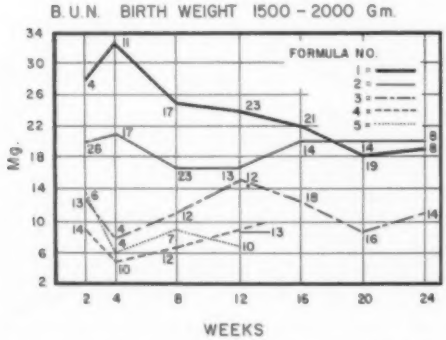

Fig. 5. Blood urea nitrogen. Birth weight 1,500 to 2,000 grams.

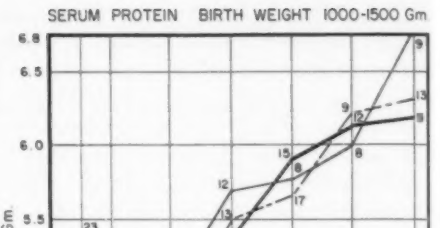

Fig. 6. Serum protein. Birth weight 1,000 to 1,500 grams.

F. Calcium and phosphorus. Serum calcium and phosphorus varied with the content of calcium and phosphorus in the formula, respectively, and not with the protein content. Pooled human milk (Formula 5), which had the lowest phosphate content, gave the highest serum calcium and lowest serum phosphorus.*

G. Urinary phenol. Urinary phenols were found to vary directly with the protein content of the diet. In all babies, except those who were fed Formula 1, phenol excretion became low at about 3 to 6 weeks. The babies who were fed Formula 1 continued to excrete large amounts of phenols as long as they remained in the nursery.

H. Food and protein efficiency. Food efficiency was calculated as the gain in weight in grams per 100 ml. of formula taken; protein efficiency as the gain in weight in grams per gram of protein (Table III). These were computed from the total amount of food ingested from the time of lowest weight of 2,250 grams. Those babies who were fed pooled human milk and Formula 4 had low food efficiency ratios. The protein efficiency ratios were consistently inversely proportional to the protein content of the formula.

*To be reported in detail.

Protein intake varied from 1.7 Gm. per kilogram to 8.0 Gm. per kilogram (Figs. 8 and 9). Food intake reached approximately 200 ml. per kilogram at about 3 weeks of age for babies who were fed each of the formulas. Although the gain in weight of the babies who were fed pooled human milk is not as good as that of those who were fed formulas with higher protein content, the good protein efficiency ratio with lowered rate of gain in weight indicates an inadequate quantity of protein in human milk for premature infants.

I. Morbidity. In this study, 4 deaths occurred in the group with the highest protein feeding (Formula 1) and none in the other groups. If these figures are added to the results of a previously reported study from this hospital[8] in which 81 babies were fed Formula 1, and 81 Formula 3, with 8 and 3 deaths, respectively, the combined death rates are 12 per 163 and 3 per 174. These differences are suggestive but not statistically significant. Morbidity was also high among the infants who received Formulas 1 and 2 (Table IV).

J. Hemoglobin. Hemoglobin levels were similar in all infants regardless of the formula given.

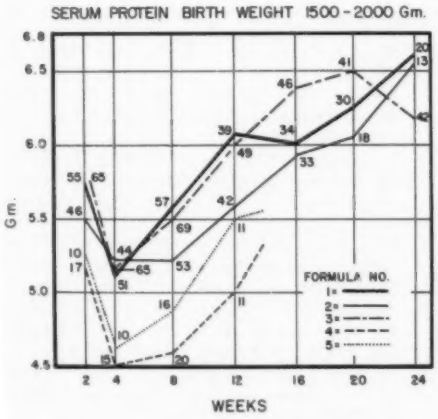

Fig. 7. Serum protein. Birth weight 1,500 to 2,000 grams.

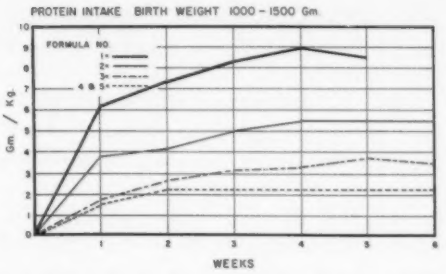

Fig. 8. Protein intake. Birth weight 1,000 to 1,500 grams.

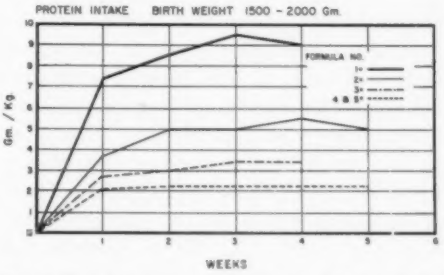

Fig. 9. Protein intake. Birth weight 1,500 to 2,000 grams.

CONCLUSIONS

The optimal protein intake of premature infants is unknown. In the study of Gordon, Levine, and McNamara,[9] premature infants who received 6 Gm. of protein per kilogram per day showed the best gains in weight. Levin and associates[10] concluded that those infants who were fed 3.5 Gm. per kilogram per day grew less well than those who received 5.5 Gm. per kilogram per day.

In the present study, the babies who were fed less than 2.5 Gm. per kilogram per day gained poorly, while those who were fed 3.0 to 8.0 Gm. per kilogram per day, gained equally well. The group of infants on the high-protein formula (1) who drank ad libitum in excess of 8 Gm. protein per kilogram per day grew less well than those taking a smaller quantity. The poor growth response of those infants on very high protein intake is suggestive of protein intoxication. We have, however, failed to duplicate this

effect in a small number of subsequent clinical trials with a different group of nurses in attendance.

The very low protein efficiency ratio for Formula 1 (4.1 per cent protein) clearly indicates that in this formula a much smaller proportion of ingested protein is used for growth gain in weight than in Formula 2, whereas the figures for the protein efficiency ratio with Formulas 3 to 5, indicate the best utilization. Final conclusions for elucidation of this finding would require analysis for total nitrogen in the body. Studies of metabolic balance are ambiguous in their interpretation.[12]

Four differences in metabolism were noted in these babies: (1) The blood urea nitrogen remained elevated for as long as 6 months in the babies who were fed more than 5 Gm. protein per kilogram per day, regardless of birth weight. (2) Stool pH was found to be directly proportional to the amount of protein in the food. (3) Phenol excretion fell to low levels in all babies except those who were fed more than 5 Gm. protein per kilogram per day. (4) Serum calcium and phosphorus varied directly with the calcium and phosphorus intake and did not appear to be related to protein intake. The similarities included: (1) Equal gains in height and weight for Formulas 1 through 3, (2) normal electrophoretic patterns, (3) no statistical differences in morbidity or mortality.

SUMMARY

Two hundred seventy-seven premature infants were fed formulas of varying composition in an attempt to evaluate growth (length and weight) and metabolic response. (The composition of the 5 formulas used is given in Table I.) Premature infants grew well for prolonged periods when fed formulas which provided 3.0 to 8.0 Gm. protein per kilogram per day. Infants fed formulas which provided more or less protein did not grow as well. Human milk formula alone did not support comparable growth of premature infants. Results obtained from feeding a formula of cow's milk with a protein content of 1.1 per cent were identical with those in infants who were fed human milk.

Infants who were fed formulas which provided over 5 Gm. protein per kilogram per day had persistently elevated blood urea nitrogen levels and excreted large quantities of urinary phenols. Other biochemical or growth differences in premature infants who were fed the other specified formulas were small.

There were no statistical differences in the serum protein values in infants who were fed 3 to 8 Gm. of protein per kilogram per day. In contrast, infants who were fed less protein have shown lowered serum protein values.

The food efficiency ratio was identical for formulas with 1.5 to 4.1 Gm. of protein and lower in those with less. In contrast, the values for the protein efficiency ratio indicate better utilization of protein for the formulas with low-protein content than for those with higher-protein content. The lowest value was found for the formula with 4.1 per cent protein.

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A procedure for hospital admission designed as a model for training residents in patient care

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PHILADELPHIA, PA.

IN A recent analysis of pediatric training, Levine[1] pointed to the necessity for residency programs to include more emphasis on developing the pediatrician's understanding of parent-child behavior. He suggested a number of ways in which the study of social, cultural, and emotional factors in child development could be introduced into the curriculum. In addition, he reviewed the radical changes in childhood illness which have produced an urgent need for emphasizing such training in pediatrics.

Since the middle 1930's, efforts have been under way to introduce this type of training into residency programs. There is increasing agreement with respect to the goals of such efforts, but methods for achieving these goals continue to be debated and the development of effective training remains a formidable task. Our own program at The Children's Hospital of Philadelphia is the outcome of many years of continued modification by both child psychiatrists and pediatricians. The aims of our program do not differ substantially from those advocated by Levine.

The special training method evolved was one of individual supervision of the resident's cases by a pediatrician specially trained in the psychological aspects of practice. The object of this supervision was to facilitate in an hour of supervisory conference each week the resident's identification of the behavioral patterns of parent and child and thereby to enable him to practice more effectively. Since repetitive exposure to the patterns of parent-child behavior would increase the possibility of recognizing the significance of such behavior, enable the resident to evaluate the effects of his own interaction with the family, and help him define his responsibilities with reference to psychosocial factors in child care, provision for continuing follow-up patient care by the resident was established in the outpatient department.

Despite this provision of follow-up cases for discussion, it appeared that cases from the inpatient service were preoccupying the residents. Reexamination of inpatient care disclosed that the majority of the patients arriving at the hospital for study and treatment were frequently confronting the physician with highly confusing clinical pictures in which the nature of child and parent behavior was distorted. As a result, the resident was becoming unfortunately involved in poorly understood problems in case management, a situation frustrating to teaching and patient care alike.

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During the "golden age of curative pediatrics," the institutional pediatrician was dealing mainly with life-threatening disease in which search for a "unit cause"[2] of disorganized function as a basis for management was both realistic and practical. In the conditions encountered commonly today, the child's development has frequently been prejudiced since birth, and the current clinical picture is the outcome of a long period of interaction between a child with defective endowment and an environment providing inadequate support for development. It follows that each case arriving at the hospital today presents a greater probability than in the past that the symptoms and behavior currently manifested by the child cannot be well understood except by consideration of the past interaction between the nature of the child and his particular nurture.

PROBLEM CASES

The problem for the residents arose from the fact that historical data initially obtained did not actually reflect the range of diagnostic probabilities resulting from the major factors influencing the current clinical picture, its course, and probable outcome. According to the common practice, there was little attempt made at the time of admission to secure more than the obvious central facts of the child's condition. More elaborate investigation was planned for a later period so that the real work-up of a case by a resident began after the child was already occupying a bed. As a consequence, the diagnostic evaluation and/or treatment frequently arrived at a frustrating impasse, the situation tending to become more confusing with each passing day in the hospital.

It was evident that the same cases disturbing the residents were causes for concern among the nurses and in the business office. A review suggested that the source of distress frequently lay in the unanticipated nature of the actual condition confronting the hospital personnel although this problem should have been anticipated from information available at the point of admission. This led to a realization that neither the training of residents nor the constructive care of child patients can be realistically carried out if the factors influencing the developmental status of the child are not considered at the beginning. These are not obtainable by the current practice of a narrow and inadequate initial assessment. For example, children with the same diagnoses such as nephrosis, rheumatic fever, etc., are frequently found to require radically different treatment plans. An effective plan of treatment must be related to the total needs of the child and the family.

It has always been recognized that illness and hospital admission present adaptive problems to parent and child. The unfamiliar and unknown aspects of illness and hospitalization have always been sources of fear and anxiety to children and these feelings cannot be allayed by the parent who is similarly affected. Where ambiguity surrounds both the illness and the procedures of the hospital, the accompanying anxiety increases. Therefore, the more that can be done to make the experience in the hospital understandable and better related to other life experience, the less the stress which will be generated by the experience per se. It must be apparent, however, that when hospital personnel themselves do not understand the real issues involved for child and family, they can only contribute to the stress rather than to its amelioration.

Some of the cases which illustrate these points may be useful to others as they were to us.

1. The child whose presence in the hospital seems to be "much ado about nothing." A 14-month-old girl was admitted to the ward on an emergency basis with a history of watery diarrhea of 3 days' duration. The child appeared to be only moderately ill. She was irritable and hungry but had only one stool in the following 3 days. The electrolyte pattern was normal.

When these findings were discussed with the referring physician on the second day of hospitalization, it became apparent that he was well aware of the anxiety inherent in this situation. He reported that the infant had been an irritable child since birth and

had consistently presented a problem in feeding. He considered the mother to be "anxious" and had felt that hospitalization was his only recourse.

Comment. This case is a typical illustration of a discrepancy between the degree of illness described by the referring physician and the physical findings actually observed following admission. The distorting factor is the intense parental anxiety associated with the child's illness. Often the referring physician reinforces the anxiety to such a degree that panic develops and admission is requested as a solution. The difficulties in later care stem from the false and paradoxical nature of the admission. In other words, while it is apparent that anxiety is the major element needing intervention, the admission is based solely on the physical symptoms. The physician is then left in the untenable position of treating only the physical symptoms while the parents are distracted from the important factors contributing to the patient's difficulty.

2. The child "unexpectedly abandoned" in the hospital. L. T., aged 16 months, was admitted to The Children's Hospital with a diagnosis of tuberculous meningitis. She had been seen on a previous occasion in the Outpatient Department for evaluation of retardation which had resulted from brain injury at birth. Tuberculosis had recently developed. She was admitted through the receiving ward when meningeal involvement became apparent. Shortly after her arrival in the ward, the mother could not be located to give a history to the house officer. Attempts by local public health authorities to get the mother to visit were fruitless.

After the patient improved and was ready for discharge, 3 additional months were required to establish a plan for future care.

Comment. This case illustrates a problem in management frequently encountered in children with chronic illness who come from families with marginal capacity to cope with such problems. The stress of the illness becomes too much for the family to bear and leads to abandonment of the child in the hospital. Frequently, the degree of family disorganization is not recognized until the time for the child to return home and often at that point re-establishment of a relationship with the parents is impossible. These cases are tremendously time consuming to the physician and are expensive to the hospital. Assessment of the sources of stress operating in the family unit is necessary before a realistic plan for hospitalization can be established.

3. Children admitted with organic disease later demonstrating severe behavioral difficulties as factors in management. C. D., aged 8 years, was transferred from another hospital to The Children's Hospital for treatment of nephrosis. Soon after admission the child presented a severe problem in management because of his aggressive behavior. It became impossible to obtain a urine sample for analysis and special nurses had to be obtained around the clock to keep the child confined to bed. The situation was disruptive for the entire ward. Interviews with the parents revealed a long history of developmental disturbance and family conflict. When the physician in the community hospital was questioned about the course of the child's previous hospitalization, it became clear that he had been unable to control the child on the ward and had for this reason, chiefly, desired transfer.

Comment. This case illustrates the consequences of failure to assess at admission all of the factors which will influence future care. The patient, indeed, did have classical nephrosis, but his management was complicated by his extremely disturbed behavior on the ward. In addition, a major problem in his future treatment quickly developed because proper follow-up care would involve separation and psychiatric treatment.

4. Psychosomatic disorders with parents' attention fixed on organic disease. A. M. was referred for admission with a diagnosis of Sydenham's chorea. Two years previously she had been thoroughly studied in another hospital and a psychological basis for her symptoms had been established. The parents refused to accept this diagnosis and the current referral for hospitalization was pre-

cipitated by the reappearance of her choreiform movements. After a brief history the admitting resident learned of the previous evaluation, but because of the aggressive behavior of the mother he elected to exclude organic disease first and to consider psychological factors later.

Study on the ward revealed the child to have a functional murmur and observation confirmed that her choreiform movements were not characteristic of classical chorea but were behavioral manifestations of an underlying emotional difficulty. Cardiac and neurological consultations supported both of these findings. Interviews produced a long history of developmental deviation, but the parents remained resistant to any suggestion that the child's symptoms were on a functional basis.

Comment. This case represents the difficulty in management created when an "either/or" dichotomy develops in the differential diagnosis at or following the time of admission for study. Too frequently in such instances differential diagnosis has been approached by first excluding organic factors and later reverting to a possible psychosocial etiology. When this course is followed the physician frequently finds himself trapped with parents who have adopted a "prove it to me" attitude. The failure to establish at admission the need for exploration of other diagnostic possibilities confronts the physician with a problem in management which increases in difficulty with the passage of time. Often psychiatric consultation or psychological testing is requested as a "rescue operation" and is accepted by the family with suspicion. Under such conditions, consultation seldom leads to parental acceptance of any constructive plan of care for the child.

ADMISSION PROCEDURE

Analysis of the management of these cases suggested that one or more of the following was lacking at admission: (1) an assessment of the needs and assets of the child and family, (2) a realistic appraisal of the ability of the institution to meet these needs, (3) an original diagnostic formulation which included psychosocial factors as etiological possibilities, and (4) an explanation to the parents of the objectives of hospitalization.

A dynamic intake process was then developed with the objective of eliminating these deficits. The method necessitates the collection of information covering the first two areas and requires interpretation to the parents of the inherent diagnostic possibilities and the purposes of hospitalization based on the information obtained.

The procedure for all ward admissions to the medical service is as follows: Whenever a request for a possible ward admission is made, the inquiry is received by a senior resident acting as an admitting officer. The inquiry may originate with an outside physician, a staff physician, the resident staff in the Outpatient Department, or a community agency. The resident at this time is responsible for examining the validity of the expectations for hospitalization. His goal is to establish a realistic basis for care incorporating the psychosocial with other factors.

To perform his task he (1) determines the referring physician's objectives in making the referral together with the family's understanding of them and assesses the validity of these objectives in relation to the needs of the patient, and (2) determines the parent's expectation of hospitalization and assesses the validity of their expectation with reference to the anticipated plan of management.

If the referral seems broadly based, appropriate to the hospital's service, and the trend in the illness is toward further physiologic disorganization, he admits the child immediately. If the basis of the referral seems related to only one facet of the case but immediate care seems necessary, again he admits the child but broadens the focus to include consideration of the developmental factors. In both of these instances it is usually impossible, because of the urgency of the situation, to conduct an exhaustive interview and there is no expectation that this be done immediately. It is required, however, that the point be made at admission that further consideration of all factors

Revised 7/12/60 THE CHILDREN'S HOSPITAL OF PHILADELPHIA Chart #_____

Division of Psychological Pediatrics

Questionnaire for Requested Ward Admissions

Date:_____ Time: _____ Number of Free Beds Available_____

Patient's Name:_____ Age:_____ Sex_____

Father's Name:_____Telephone:_____

Address_____

Referring Physician or Agency:_____ Phone:_____

Address:_____

Referring Diagnosis or Problem:_____

_____

Admitting Resident's Initial Impression:_____

Expectations for CHOP:

of Referring Doctor:_____

_____

of Family:_____

_____

Is Emergency Admission Recommended?

By Referring Doctor:_____ Why? _____

_____

By Admitting Resident:_____ Why?_____

_____

Responsibility for Patient:

Custody on Admission:_____

Custody after Discharge:_____

Transportation to and from CHOP:_____

Hospital Expenses:

Estimate of status. Full Pay____ Part Pay____ Free____ Insurance_____

Medical follow-up after discharge_____

Disposition:

_____ Admitted as Emergency

_____ Consultation with Supervisor of Admissions

_____ Deferred CHOP Admission

Date of Admission_____

_____ Deferred OPD Work-Up

Clinic Appt. Date:_____ Time:_____

Resident_____

File Opened?_____

_____ Seen in Receiving Ward

_____Referred to other source:

_____

_____Further information requested.

_____Referred to Admin. Asst. OPD

Signed:_____, M.D

Asst. Chief Resident Physician

Fig. 1

will be a part of future management and that parental involvement in this task will be necessary.

If the referral seems to be based on factors which do not warrant immediate admission, e.g., undue anxiety on the part of the

family or the wish to be rid of the case on the part of the physician, he defers admission pending further evaluation. Hospitalization is neither agreed to nor denied, and the admitting officer explains that the purpose is to understand the family and child's needs more fully and so to establish a sound basis for hospitalization if this is necessary. If the family or referring physician is concerned over the delay, it is made clear to them that the hospital is available at all times for reconsideration of admission. Often, when the admitting officer holds to the need for further evaluation and planning, this in itself raises many significant questions for the parents, makes the next contact more productive, and promotes consrtuctive parental involvement in potential hospitalization.

At the time of inquiry the admitting resident fills out a form (Fig. 1) indicating his understanding of the needs of the case and his concept of the plan of management required to meet these needs. This form is devised to record the following:

1. The resident's assessment of the needs of the case.
2. The referring physician's assessment of the needs of the case.
3. The referring physician's objectives in making the referral and the family's understanding of these.
4. The family's and referring physician's expectations of the hospitalization.
5. The resident's understanding of the purposes of admission.

This type of form is valuable since it requires the collection of standardized information validating the request for admission and requiring the admitting officer to justify his course of action.

INTAKE REVIEW

In The Children's Hospital there are four senior residents who rotate responsibilities for admission to the ward service. Each of these house officers and the authors attend a weekly Intake Review Conference. At this conference the admitting officer reviews the form and presents a history of the admission of each case. The resident in charge of the patient on the ward then reports on the problems in management as seen during hospitalization. The purpose of the conference is for the group to examine the steps taken at admission and to compare them with the subsequent course of management. From this experience the admitting officers learn the outcome of their efforts and are helped to apply this knowledge more effectively the next time a similar problem is encountered. Psychosocial factors are discussed only if such factors will facilitate subsequent management. These conferences have been a fruitful experience for all participants over the past year and a half and the knowledge gained has led to constant modification of the intake process.

Interpersonal problems with referring physicians, agencies, and other hospitals are discussed and ways of resolving them are explored. If differences arise involving professional relationships with these sources of referral, they are handled at a staff level through consultation with the referral source.

RESULTS

The preliminary results of the application of these procedures have been encouraging. While we have no statistical proof, our general impressions have been that this approach to admission has resulted in improved ward care. There have been no "abandoned children" on the ward service for the past 8 months and the incidence of ward patients remaining in the house over 30 days has significantly decreased. The ward patients still have the same kinds of problems, but with a "broadened admission contract" an improved basis for management has resulted. The residents' interest in and utilization of the principles inherent in this approach to intake have steadily increased. Relationships with referring physicians and other resources have been difficult but as the skill of the resident in handling admissions has increased, understanding and acceptance of the purpose of the intake process by the referring physician has followed.

The following is a case illustrating ap-

plication of a broadened approach to admission:

Illustrative case. D. B., aged 3, was brought to the accident ward in semicoma following the ingestion of several capsules of a tranquilizer which had been prescribed for his mother. When the admitting resident obtained this history and examined the child, he indicated to the mother that hospitalization would be necessary for proper treatment. The mother's first reaction was one of acceptance but this was followed shortly by extreme agitation. She began to cry, "Please don't let my child die," and then experienced a brief episode of syncope. As she revived, the resident assured her that the child would probably be all right but then expressed his concern about the intensity of her fears. She revealed that within the past 2 weeks her father and her maternal aunt had died. The stress of these events had made her so anxious that her physician had prescribed a tranquilizer and had advised her to rest as much as possible. She stated that during this time the patient became more active and began to be excessive in his demands on her. He had always been a hard child to manage but in these past 2 weeks he had become almost unbearable.

With this additional information the resident broadened the purposes of the hospitalization to include help for the mother in relation to these additional factors. He responded to her concern by explaining that the current stress and her past difficulties with child rearing were very relevant to the patient's ingestion of the tranquilizer and explained to her that these problems could be explored with her by the physician who would care for the patient in the ward. She welcomed this opportunity.

The ward physician was then introduced to the mother and the foregoing objectives of the hospitalization were outlined to him. Immediately following admission the mother talked freely about her past and present involvement with the patient. She stated that she was anxious to understand why this problem had arisen and what might be done to prevent its recurrence. The child's history disclosed a chronic pattern of maladaptive behavior characterized by periods of excessive dependency, irritability, and hyperactivity. Repeated episodes of pica had been a constant problem to the mother. As she discussed her concerns about the child she began to understand that the ingestion of the tranquilizer was the latest manifestation of a long-standing problem in the child's development. Observations of the patient on the ward confirmed the findings in the history. The patient gradually responded to forced fluids and was completely conscious within 24 hours. His subsequent behavior was characterized by extreme activity while out of bed and excessive thumb sucking when confined. Urine examination for coproporphyrin was negative.

In spite of the facts that there were six siblings at home, ranging in age from 14 years to 6 months, and the mother was separated from her husband, she made frequent visits to the ward. She began to resolve any question of guilt about having contributed to the child's current illness through her own neglect. Her interest became focused on understanding the child's developmental difficulties. A follow-up program was outlined including social service and referral to the Child Guidance Clinic with continuous pediatric follow-up.

Comment. This case illustrates the possibilities for care when a basis for treatment is outlined at intake. In the Case Review Conference which followed this admission, the ward staff was unanimous in its agreement that the objectives as outlined at admission were accomplished. The hospitalization was brief (8 days) and the goals of care were clear to the staff and to the patient's mother. The hospital course was comparatively smooth and the management of the case rewarding. It was felt that the clarity established at the time of admission accounted for this success.

DISCUSSION

The intake procedure described is based on a model in which the goal of pediatric

care is the promotion of child development. The resident is taught that illness is a contingency in the continuum of development and that the responsibility of the physician in treating a sick child is to perform his task in support of development. Separation from home and the fear of loss of function through illness always accompany hospitalization. Thus, they present a hazard to further maturation. The capacity of the child to cope with such an experience depends on the nature of the supports available to him from his past experience, his family, and his medical caretakers. The quality of the medical supports, therefore, may become a critical factor in the outcome of the child's developmental progress. At the time of hospitalization of the child, parents frequently review their past and reconsider plans for the future and are amenable to change in a unique way. The intake procedure is timed to capture the dynamic elements of this situational crisis that occurs on admission. Critical situations of this nature have been discussed extensively by Rose,[3] Caplan,[4, 5] and others[6] as hazardous circumstances which may lead either to developmental regression or to increased capacity for adaptation. Medical care which is sensitive to this opportunity may be mobilized to support a constructive change in the trends of the child's adaptability.

We believe that the procedure of admission has significance for all areas of pediatrics. At the time a well child is seen and a new family is being admitted to a private practice, it is clearly necessary to make an assessment of the family in order to determine whether the pediatrician can supply the services required and to define his role in relation to the child's development. Similarly, when a sick child is seen by a physician in an outpatient department, private office, or community hospital it is also necessary to assess the total needs of the child and the family and to establish a plan of care which will support continuing growth. Emphasis on the admission procedure as part of residency training should provide the physician with valuable skills which will have broad application in his practice.

SUMMARY

Increasing numbers of patients with complex and chronic illnesses are being admitted to pediatric hospitals. The problems of clinical management of these patients are difficult because of the multiplicity of factors contributing to the patient's dysfunction. The assumption has been made that many problems in ward management and pediatric training are the result of inappropriate acceptance of these patients since admission is often effected on the basis of a single facet of a complicated disorder. Relevant case illustrations have been presented.

A different approach to admission, which establishes a broad basis for future management, is described. A case illustrating this approach is presented and a model of care centered on child development is offered as a basis for understanding the dynamics of this intake process.

The conclusion drawn is that the use of this admission's procedure has resulted in an improvement of both care and training. Reference is made to its significance for pediatric practice.

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*News and notes**

A course in **Pediatric Neurology** is to be presented Feb. 12 to 14, 1962, at the Center for Continuation Study, University of Minnesota. Address inquiries to the Director, Department of Continuation Medical Education, 1342 Mayo Memorial, University of Minnesota, Minneapolis 14, Minn.

Yale University School of Medicine Cystic Fibrosis Center announces a **Fellowship in Cystic Fibrosis,** of either 1 or 2 years' duration, open to candidates with, preferably 2 years of pediatric training. Emphasis will be on intensive clinical studies and clinical research with respect to the relationships between basic disturbances in acid-base balance and electrolyte distributions and pulmonary disease in cystic fibrosis. Address Dr. Vivian Tappan, Director, Cystic Fibrosis Program, Yale University School of Medicine, 333 Cedar Street, New Haven 11, Conn.

The Clinic of Child Development of The Children's Hospital of Columbus, Ohio, and the Department of Pediatrics of Ohio State University College of Medicine offer positions and training in **Developmental Pediatrics and Infant Neurology.** Starting salary for those who have completed a 2-year residency in pediatrics is $8,640.00; more advanced status is available if the applicant is certified by the Board; American citizenship, or intended citizenship, is a requirement. Address Dr. Hilda Knobloch, Associate Professor of Pediatrics, Children's Hospital, Columbus 5, Ohio.

The Department of Pediatrics of Emory University announces a **postgraduate course** entitled **Review of General Pediatrics,** to be held on March 12 to 14, 1962, at the Grady Memorial Hospital and Egleston Hospital for Children in Atlanta, Georgia. Guest faculty will be Dr. Heinz Eichenwald of Cornell, and Dr. Richmond Paine of Boston. Address W. G. Thurman, M.D., 69 Butler Street, S.E., Atlanta 3, Ga.

A 2-week **Postgraduate Course in Allergy** is to be offered by the Departments of Allergy and Applied Immunology of the Temple University Medical Center and the Graduate School of Medicine of the University of Pennsylvania. Sessions will be conducted at Temple daily from March 5 to 16, 1962. Tuition fee is $175.00, and enrollment is limited. Address Dr. George Blumstein, Temple Medical Center, Philadelphia 40, Pa.

*Notices of meetings must be in the hands of the Editor 3 months in advance of the date in order to appear in the JOURNAL in time.

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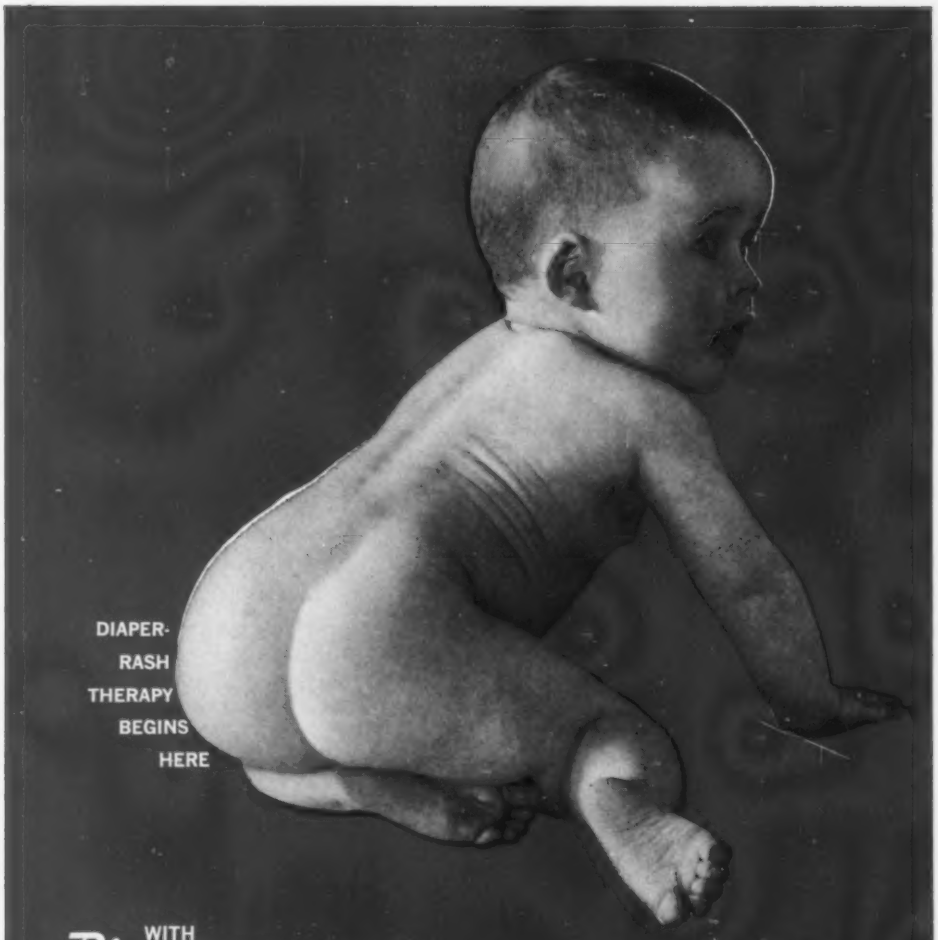

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MOUTH OF CHILD, AGE 11

prophylaxis
against tooth decay

THE NUMBER "ONE" CHRONIC DISEASE

should be included in
pediatric medical care

teeth can be made more resistant to decay with daily dietary fluoride during infancy and childhood...

"Most dental decay can be prevented. More than 30 years of scientific research has proved that fluoridated water, drunk from infancy, prevents 2 out of 3 cavities in children's teeth and that it continues to be effective through life."(1)

The importance of dietary fluoride in increasing the resistance of teeth to decay and preventing dental caries was underscored in a recent report issued by a Joint Committee of the AMERICAN ACADEMY OF PEDIATRICS and THE AMERICAN SOCIETY OF DENTISTRY FOR CHILDREN: **"To achieve maximal caries-preventive effect, fluoride should be ingested during that time when the teeth are in the formative stage and throughout the caries-susceptible years. This ingestion must cover a period from the fourth month *in utero* (when the first deciduous central incisors begin to calcify) to the age of 18 years."**(2) They concluded their report with: "As dental caries is primarily a disease of childhood and appears to be at least in part preventable, the pediatrician is obliged to be interested in this problem and can play an important part in prophylaxis."

Although fluoridated drinking water is the most practical and dependable procedure in the prevention of dental caries . . . only 25% of our population benefits from fluorides in their drinking water. Even if all central water supplies were fluoridated, approximately 50% of our people would not have fluoridated drinking water at their disposal. Therefore, when not available, fluoride supplementation is most desirable. In this connection, the AMERICAN DENTAL ASSOCIATION states: **"On an *a priori* basis it may be assumed that daily dietary supplements of 1 mg. of fluorine in the form of sodium fluoride are desirable for young children (under 8 years of age) living in rural areas or other localities where community fluoridation progams are not feasible. However, such administration should be consistent and continuous over long periods of time if substantial benefit is to be anticipated."**(3)

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1. Ephedrine Sulphate 12 mg. — for decongestion and relief of bronchospasm
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4. Glyceryl Guaiacolate 50 mg. — for effective expectorant action
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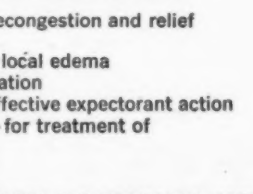

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P. E. Conen, M.S., B.S., M.R.C.P.(London), A. G. Bell, M.D., and C. P. Rance, M.C., F.R.C.P.(C), Toronto, Ont.

Spontaneous rupture of the esophagus in the newborn
Van D. Chunn, Captain, MC, USA, and Leo J. Geppert, Colonel, MC, USA

Neonatal sciatic palsy after umbilical vessel injection
M. San Agustin, M.D., H. M. Nitowsky, M.D., and Jesse N. Borden, M.D., Baltimore, Md.

"Idiopathic" hematuria
L. B. Travis, M.D., C. W. Daeschner, M.D., W. F. Dodge, M.D., H. C. Hopps, M.D., and H. S. Rosenberg, M.D., Galveston, Texas

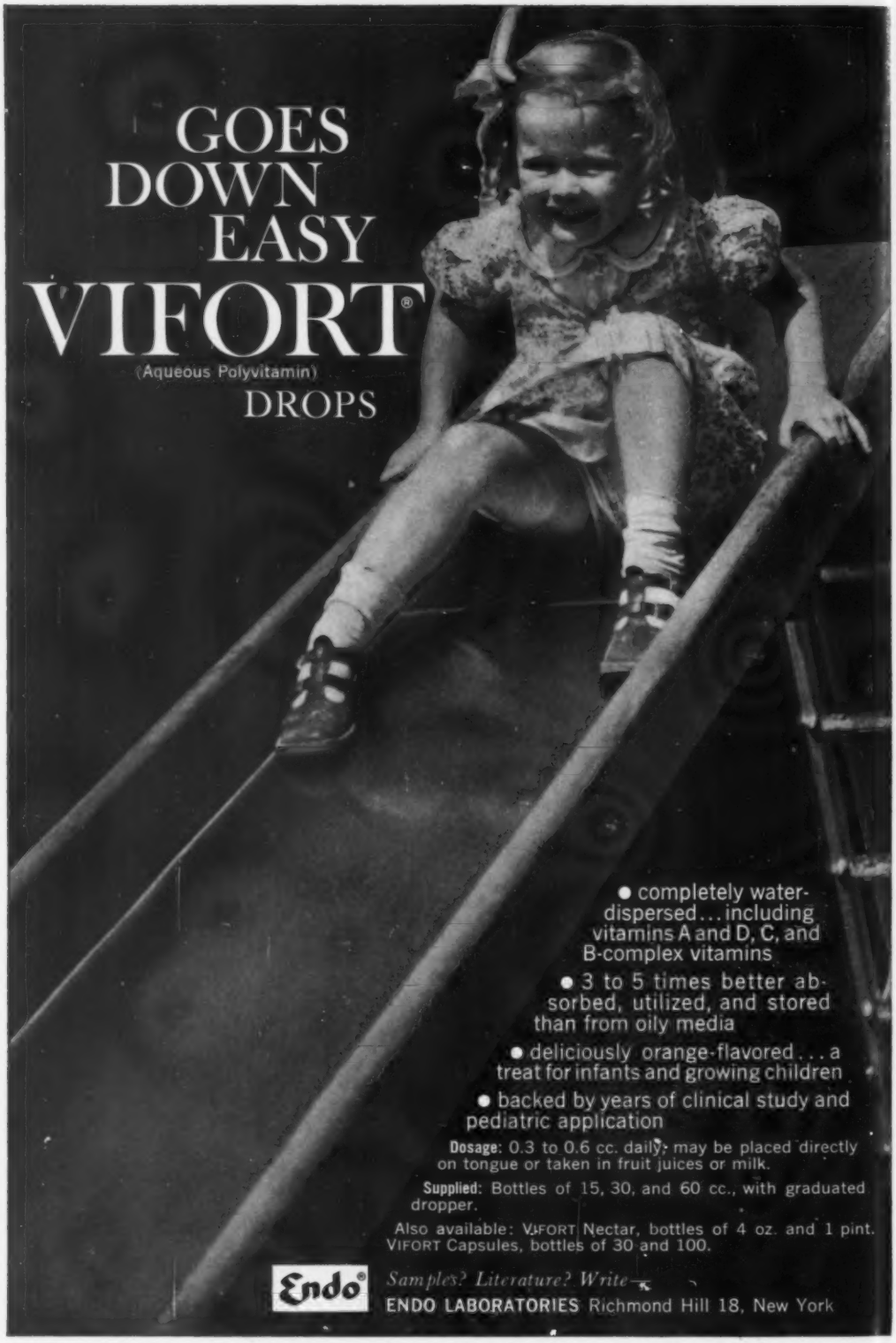
Pyrvinium pamoate in the treatment of pinworm infection (enterobiasis) in the home
Jerrold A. Turner, M.D., and Paul E. Johnson, Jr., M.D., Los Angeles, Calif.

Livedo reticularis: A form of allergic vasculitis
W. D. Bradford, M.D., C. D. Cook, M.D., and G. F. Vawter, M.D., Boston, Mass.

Muscular dystrophy in infancy
Anthony J. Lewis, M.B., B.S., D.T.M.&H., Dip.Path., and Donald F. Besant, M.D., Winnipeg, Man.

Studies in sickle cell anemia. XIX. Priapism as a complication in children
Consuelo M. Sousa, M.D., Bette L. Catoe, M.D., and Roland B. Scott, M.D., Washington, D. C.

Vitamin A deficiency in African children in Northern Rhodesia
Bent Friis-Hansen, Dr. med., and Fergus S. McCullough, Ph.D., Brassaville, Northern Rhodesia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References: (1) Vignec, A. J., and Gasparix, M.: J.A.M.A. 167:1821 (Aug. 9) 1958. (2) Current Concepts in Therapy: New England J. Med. 257:513 (Sept. 12), 1957. (3) Smith, P. K., in Drill, V. A.: Pharmacology in Medicine, New York, McGraw-Hill Book Company, Inc., 1954, pp. 20/16, 20/17. (4) Hart, E. R.: J. Pharmacol. & Exper. Therap. 89:205, 1947. (5) Litter, M.; Ruiz Moreno, A., and Donin, L.: Ibid. 101:119, 1951. (6) Salamon, S. D.: Correspondence, J.A.M.A. 160:703 (Feb. 25) 1956. (7) Prien, E. L., and Walker, B. S.: J.A.M.A. 160:355 (Feb. 4) 1956. (8) Prien, E. L.: GP 15:80 (Feb.) 1957. (9) Mandel, H. G.; Redwell, V. W., and Smith, P. K.: J. Pharmacol. & Exper. Therap. 106:433, 1952. (10) Quick, A. J., and Ciesceri, L.: Ibid. 128:95, 1960. (11) Seeberg, V. P.; Hansen, D., and Whitney, B.: Ibid. 101:275, 1951.

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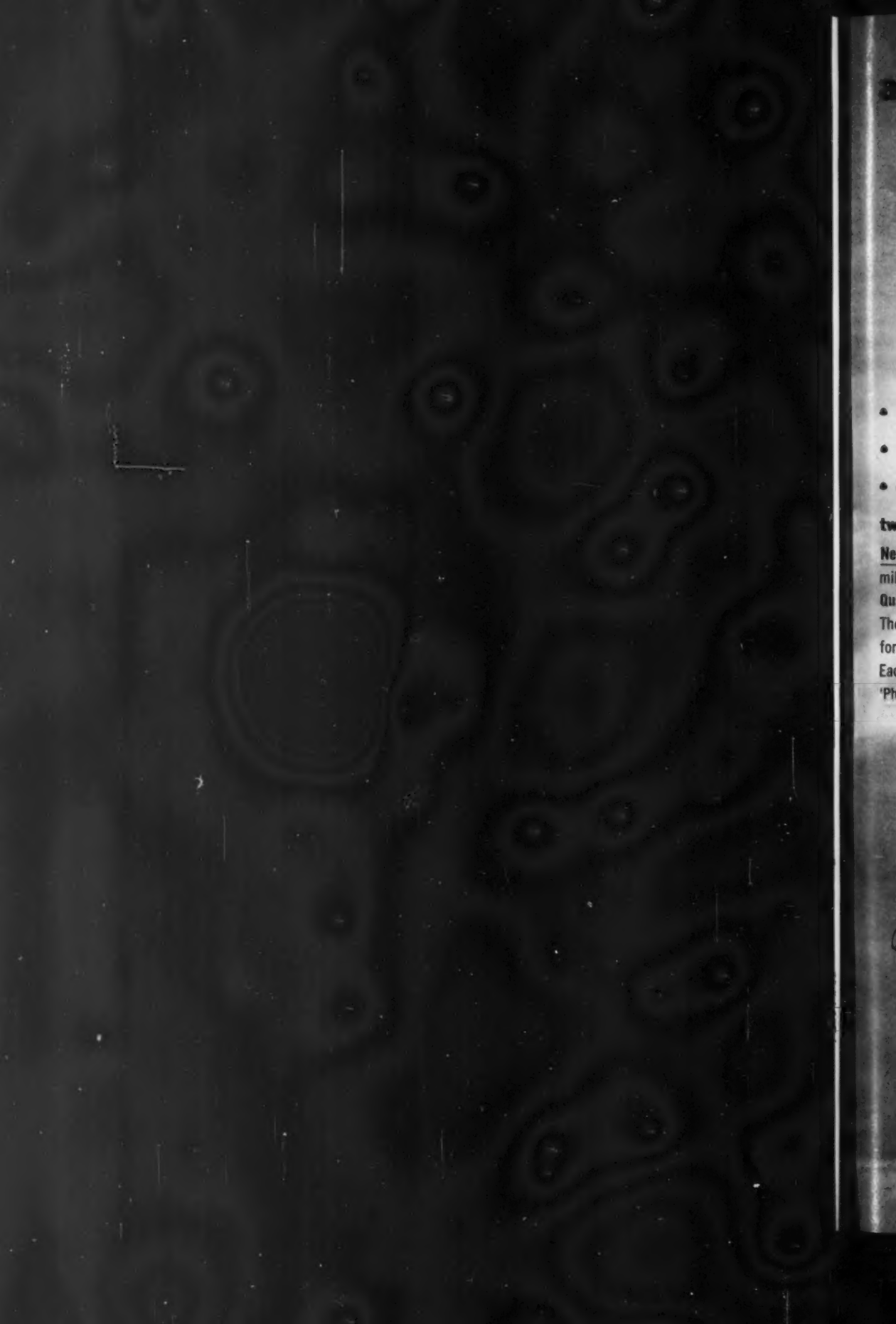

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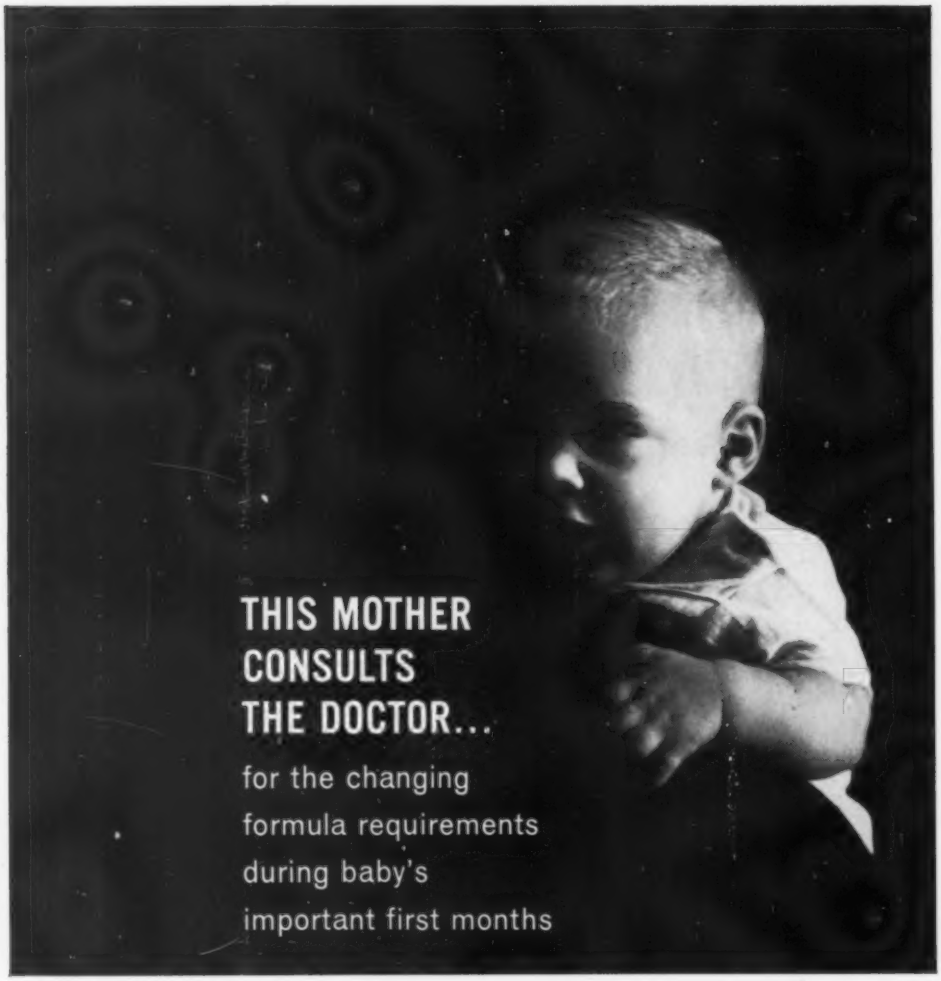

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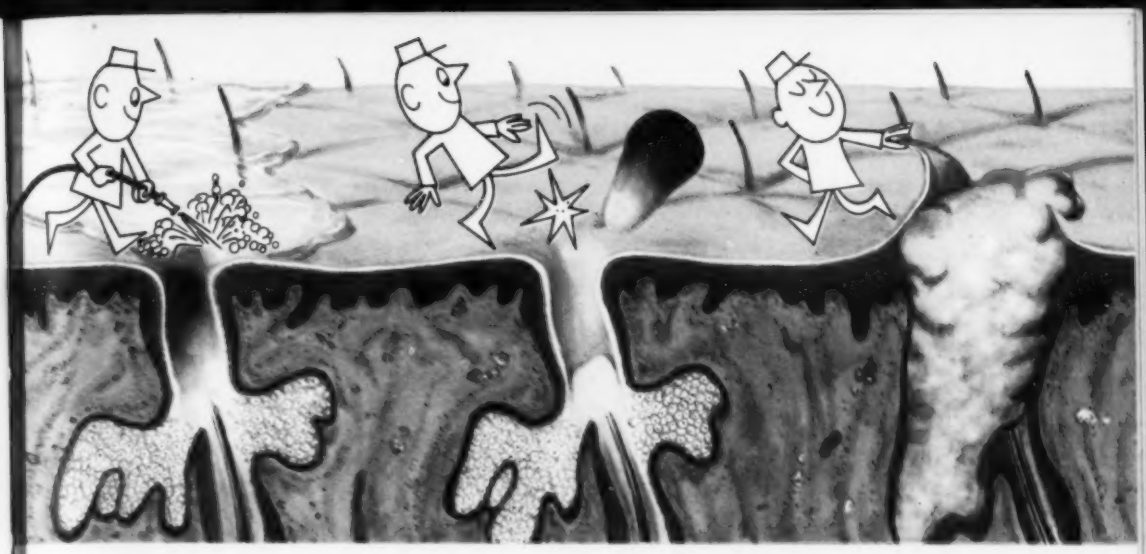

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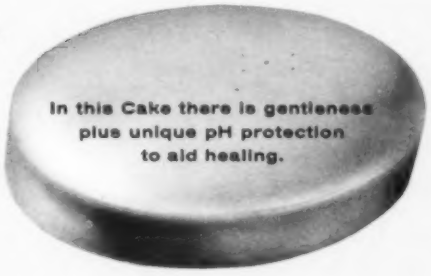

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*Nelson, L. S., and Stoesser, A. V.: Ann. Allergy *11*:572-579 (Sept.-Oct.) 1953.

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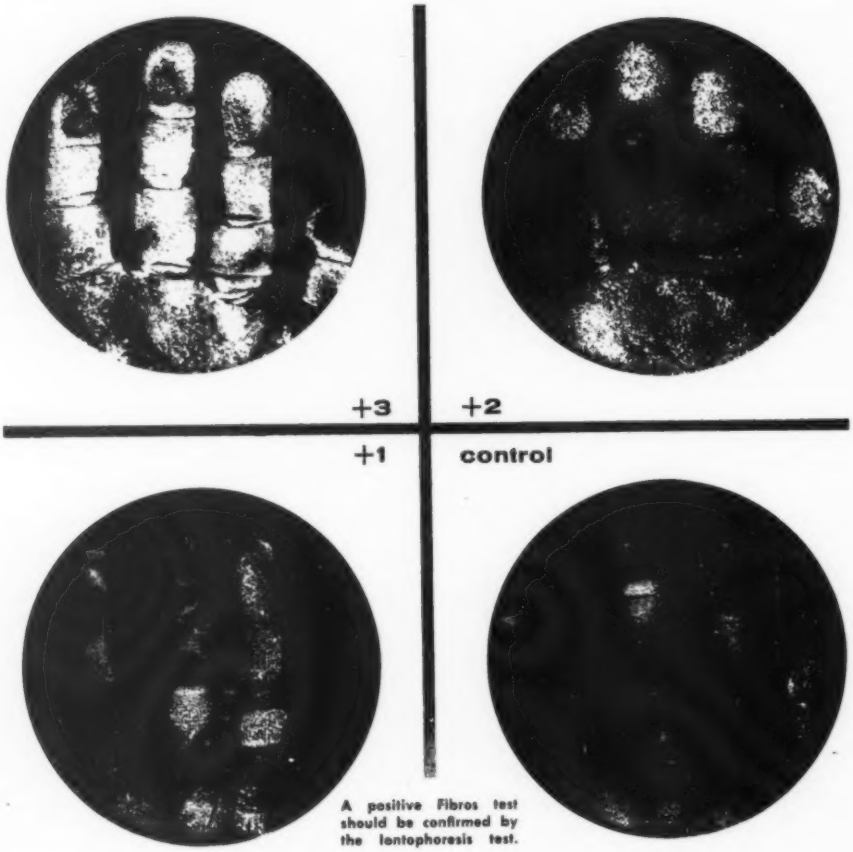

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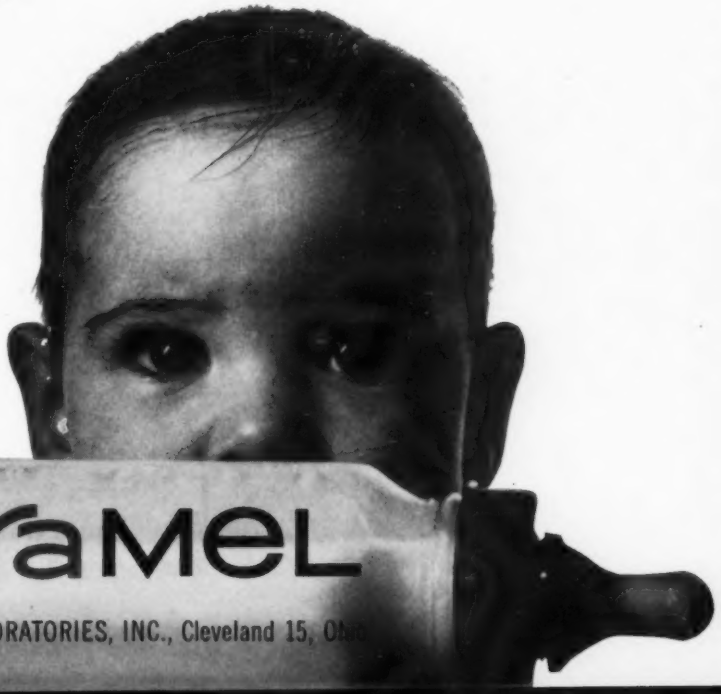
Varamel, because it contains no butterfat and no added carbohydrate, makes an excellent diagnostic aid in solving cases where milk allergy is suspected. It often eliminates the need for placing the infant on a costly milk substitute, and helps to allay the mother's anxiety over her supposed milk-allergic baby.

Since Varamel is a flexible formula, the physician has complete control of the addition of carbohydrate and water.

Varamel is unique: made only from Grade A milk, contains an optimum protein level, the R.D.A.[2] of iron and all essential vitamins, no butterfat, and no added carbohydrate.

OPTIMUM NUTRITION
Providing all the normal dietary requirements plus a reserve for stress situations.



1. Breslow, Lawrence: J. Ped. 50: 196 (1957)

2. Recommended Dietary Allowances, NAS-NRC Publ. 589 (1958)

They Bounce Back Fast

with TYLENOL®

acetaminophen

antipyretic / analgesic

He's the same young patient who was feverish and listless yesterday—Tylenol quickly brings fever and discomfort under control.

Tylenol is safe, exceptionally free from side effects[1,2]...well tolerated by children.[1]

AFTER T&A, the gentle antifebrile-analgesic action of Tylenol reduces restlessness, aids recuperation.

TYLENOL ELIXIR—120 mg. (2 gr.) per 5 cc.; 4 and 12 fl. oz. bottles
TYLENOL DROPS—60 mg. (1 gr.) per 0.6 cc.; 15 cc. bottles with calibrated droppers

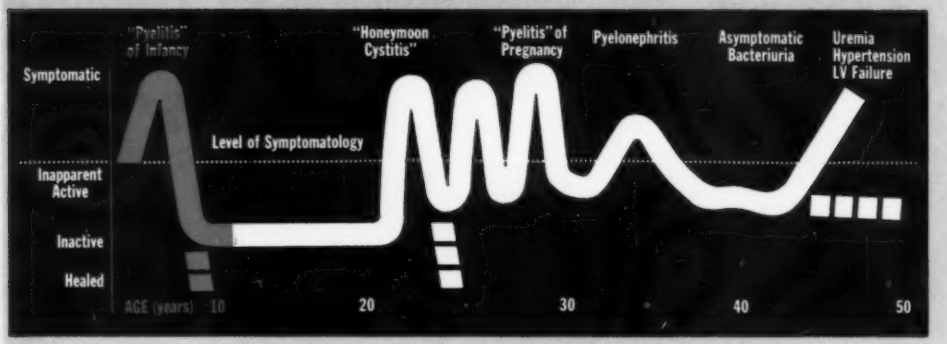
For adults and older children: TYLENOL TABLETS—5 gr. (300 mg.)

McNEIL

McNeil Laboratories, Inc., Fort Washington, Pa.

1. Cornely, D.A., and Ritter, J. A.: N-acetyl-p-aminophenol (Tylenol Elixir) as a Pediatric Antipyretic-Analgesic. J. A. M. A. 160:1219-1221 (April 7) 1956.
2. Mintz, A.A.: Management of the Febrile Child. J. Ky. Acad. Gen. Prac. 5:26-31 (January) 1959.

Natural History of Pyelonephritis



". . . the theme that runs through the carefully taken history of most uremic patients with chronic pyelonephritis—the burning on urination of infancy, the chills and fever in childhood, the 'honeymoon' pyelitis, the recurrent urethritis treated so well and often locally—and yet the termination in uremia."[1]

in early childhood—"a potentially fatal warning sign"[2]

The *best* opportunity to eradicate urinary tract infection (and prevent potentially disastrous sequelae) is the *first* opportunity—in the infant and young child.

Furadantin—for a "cure" instead of a "chronic"

In children "a prophylactic regimen of therapy is indicated. . . . The therapy could be compared to the prophylactic treatment of patients whose exacerbation of a rheumatic fever has been controlled."[3] "Continuous prophylactic therapy with nitrofurantoin, at present, is our best modality for the treatment of chronic urinary tract infection."[4]

FURADANTIN DOSAGE FOR CHILDREN: Average dose is 5 to 8 mg. per Kg. (2.3 to 3.6 mg. per lb.) in 4 divided doses daily. A prophylactic dosage of from 1 to 5 mg. per Kg. is recommended for long-term use.[3] After the infection has been controlled, urinalysis and culture at least twice a year are suggested.[3]

SUPPLIED: Oral Suspension, 25 mg. per 5 cc. tsp., readily miscible with water, infant formulas, milk or fruit juices. Tablets, 50 mg. and 100 mg.

REFERENCES: 1. Birchall, R.: Am. Practit. *11*:918, 1960. 2. Stevenson, S. S.: J. Louisiana Med. Soc. *110*:219, 1958. 3. Marshall, M., Jr.: J. Kentucky Med. Assoc. *59*:35, 1961. 4. Johnson, S. H., III, and Marshall, M., Jr.: J. Urol. *82*:162, 1959.

Complete information in package insert or on request to the Medical Director.

EATON LABORATORIES, Division of The Norwich Pharmacal Company, NORWICH, NEW YORK

To cure or control infection throughout the urinary system at every age of life... at every stage of infection

Furadantin®

brand of nitrofurantoin

total coverage of the cold syndrome.

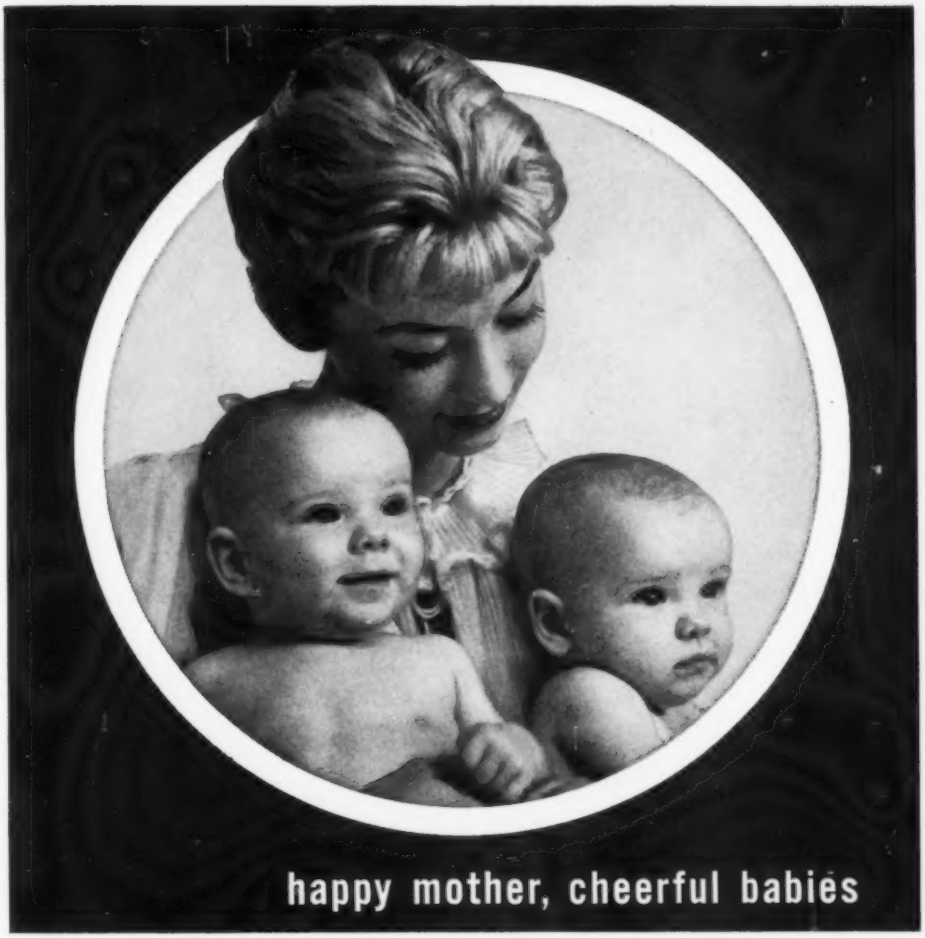
TUSSAGESIC provides TRIAMINIC®, superior upper respiratory decongestant for the stuffed and running nose. DORMETHAN®, non-narcotic antitussive and TERPIN HYDRATE, classic expectorant for cough. APAP, effective antipyretic-analgesic for pain and fever.

Tussagesic®

SUSPENSION

Each tsp. (5 ml.) of Tussagesic provides: TRIAMINIC 25 mg. (phenylpropanolamine hydrochloride, 12.5 mg., pheniramine maleate, 6.25 mg., pyrilamine maleate, 6.25 mg.); DORMETHAN (brand of dextromethorphan HBr) 15 mg.; APAP (acetaminophen) 120 mg.; TERPIN HYDRATE, 90 mg. **DOSAGE** (every 3 or 4 hours): children 6 to 12, 1 tsp.; children 1 to 6, ½ tsp.; children under 1, ¼ tsp. Also available: Tussagesic timed-release tablets for patients over 12 — 1 tablet every 8 hours.

DORSEY LABORATORIES · *a division of The Wander Company* · **Lincoln, Nebraska**



because their physician has kept the twins well nourished, healthy, and

free from diaper rash

with

DESITIN®
OINTMENT

Protects against irritation of urine and excrement; markedly inhibits ammonia-producing bacteria; soothes, lubricates, stimulates healing.

For samples of Desitin Ointment, pioneer external cod liver oil therapy, write...

DESITIN CHEMICAL COMPANY
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Also available:

DESITIN HC OINTMENT with Hydrocortisone (¼% or 1% Hydrocortisone) — anti-inflammatory, antipruritic steroid enhanced by the soothing, healing Desitin formula to control inflamed, itchy, eczematous and allergic skin conditions.

NOW! 31% PRICE REDUCTION ON DESITIN HC OINTMENT WITH HYDROCORTISONE 1%

Forhistal® Syrup

Next time you treat a youngster for allergy or itch, try Forhistal. Jacques and Fuchs* reported good to excellent results with Forhistal in 84 per cent of the children in their study. Forhistal Syrup has these special advantages: It is slightly sweet, but without distinct flavor, and has no local anesthetic effect. Children—even those with finicky tastes—accept it readily.

SUPPLIED: *Syrup* (pink), containing 1 mg. Forhistal maleate per 5-ml. teaspoon. *Pediatric Oral Drops* (pink), containing 0.5 mg. Forhistal maleate per 0.6 ml. *Tablets*, 1 mg. (pale orange, scored). *Lontabs*, 2.5 mg. (orange).

For complete information about Forhistal (including dosage, cautions, and side effects), see current Physicians' Desk Reference or write CIBA, Summit, N.J.

*Jacques, A. A., and Fuchs, V. H.: J. Louisiana M. Soc. *113*:110 (March) 1961.

FORHISTAL® maleate (dimethpyrindene maleate CIBA)
LONTABS® (long-acting tablets CIBA)

CIBA
Summit, N.J.

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1 2

3 4 5 6 7 8 9

virtually without rebound

as long as most colds last

24/31 25 26 27 28 29 30

TYZINE®

BRAND OF TETRAHYDROZOLINE HYDROCHLORIDE (0.05%)

for nasal congestion

"The 'fatigue' phenomenon, in which the nasal congestion no longer responds after frequent use of nose drops over a prolonged period, was not encountered with Tyzine solution, even in patients using it regularly for as long as two weeks."

Menger, H. C.: New York State J. Med. 55:812, 1955.

PEDIATRIC NASAL DROPS

IN BRIEF

TYZINE is tetrahydrozoline hydrochloride, a sympathomimetic amine with potent decongestant properties. Relief is almost immediate and lasts four to six hours after a single administration. Virtually free of sting or burn and rebound congestion . . . odorless and tasteless. TYZINE is not significantly absorbed systemically when used as directed . . . does not impair ciliary activity . . . and is physiologically buffered to pH 5.5.

INDICATIONS: Relieves inflammatory hyperemia and edema of the nasal mucosa and congestive obstruction of sinus and eustachian ostia, as may occur in the common cold, hay fever, perennial vasomotor rhinitis, chronic hypertrophic rhinitis, and sinusitis.

DOSAGE AND ADMINISTRATION: Infants under 2 years – 1 or 2 drops. Children 2 to 6 years – 2 or 3 drops. Instill in each nostril as needed, not more often than every three hours.

SIDE EFFECTS: Transient mild local irritation after instillation has been reported in rare instances.

PRECAUTIONS: Always use the 0.05% pediatric strength for infants and younger children. The 0.1% concentration of TYZINE should be restricted to adults and children 6 years and over. Avoid doses greater or more frequent than those recommended above. Use with caution in hypertensive and hyperthyroid patients. Overdosage may cause drowsiness, deep sleep, respiratory depression, marked hypotension or even shock in infants and young children. KEEP OUT OF HANDS OF CHILDREN OF ALL AGES.

SUPPLIED: TYZINE Pediatric Nasal Drops, ½-oz. bottles, 0.05%, with calibrated dropper. Also available: TYZINE Nasal Solution, 1-oz. dropper bottles, 0.1%. TYZINE Nasal Spray, 15 cc., in plastic bottles, 0.1%.

More detailed professional information available on request.

Science for the world's well-being® Pfizer PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. New York 17, New York

This COMPLETE formula protects your babies against butterfat intolerance

- assures optimal caloric efficiency
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These advantages in Baker's Modified Milk result from complete butterfat replacement with controlled amounts of coconut and corn oils.

Since the infant's health depends upon total adequacy of his diet, Baker's Modified Milk also provides an optimum protein level (3.7 gms/kg of body weight/day), 7% carbohydrate as multiple sugars, and the Recommended Daily Allowances of vitamins and iron.

That is why more and more physicians, and hospitals, specify Baker's Modified Milk. It is complete... at low cost per feeding. Made only from Grade A Milk—liquid or powder.

OPTIMUM NUTRITION—Providing all the normal dietary requirements plus a reserve for stress situations, while avoiding the hazards of excessive amounts of individual nutrients.

REFERENCES: Jeans & Marriot: Infant Nutrition, Ed. 4, Mosby (1947) • Adam, Doris J.D., et al: J. Nutrition 66:555 (1958) • Hansen, A.E., et al: J. Nutrition 66:565 (1958) • Recommended Dietary Allowances, NAS-NRC Publ. 589 (1958) • U.S.P.H.S. Milk Code, Federal Security Agency Publ. 220 (1953)

Baker's MODIFIED MILK

is scientifically formulated to duplicate the nutritional results of human milk.

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because it's so easy to give...and so easy on the patient

Children offer little or no resistance to this enema unit specifically designed for pediatric use. The compact FLEET ENEMA PEDIATRIC avoids the discomfort of large volumes of liquid...takes only a minute. Insertion is made easier and safer because of the pre-lubricated, anatomically correct 2-inch rectal tube.[1] FLEET ENEMA PEDIATRIC can be prescribed with confidence as "a safe and effective enema preparation for even small children."[2]

FLEET ENEMA *Pediatric*, 2¼ fl.oz. *Regular* FLEET ENEMA, 4½ fl.oz. *Each 100 cc. contains* 16 Gm. sodium biphosphate and 6 Gm. sodium phosphate. *Also available:* FLEET® OIL RETENTION ENEMA, 4¼-fl.oz. ready-to-use unit containing Mineral Oil U.S.P.

1. Frech, H. C., and Lanier, L. R., Jr.: Am. J. Obst. & Gynec. 74:1146, 1957. 2. Way, W. G., et al.: Virginia M. Month. 85:291. 1958.

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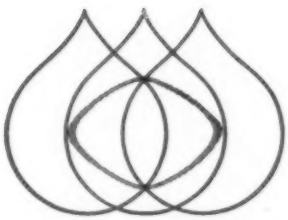

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all forms of TRI-VI-SOL®, POLY-VI-SOL®, and DECA-VI-SOL® vitamins—to provide rational, practical, safe levels of vitamins C, D, and A to fit the needs of today's children

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VI-SOL® VITAMINS

TRI-VI-SOL® · POLY-VI-SOL® · DECA-VI-SOL®

newly reformulated to complement the dietary patterns of today's infants and children

Beginning with the critical newborn period and continuing through the preadult years, new Vi-Sol vitamins supplement and complement the dietary patterns of today's infants and children. They are formulated with consideration of the recommendations of the authorities* and of the practical needs of everyday medical practice.

Vi-Sol vitamin drops provide conservative yet assured protection as the baby progresses from formula feeding to solid food. When drops are outgrown, comparable improvements in levels of C, D, and A are now offered the older child in Vi-Sol chewable vitamins.

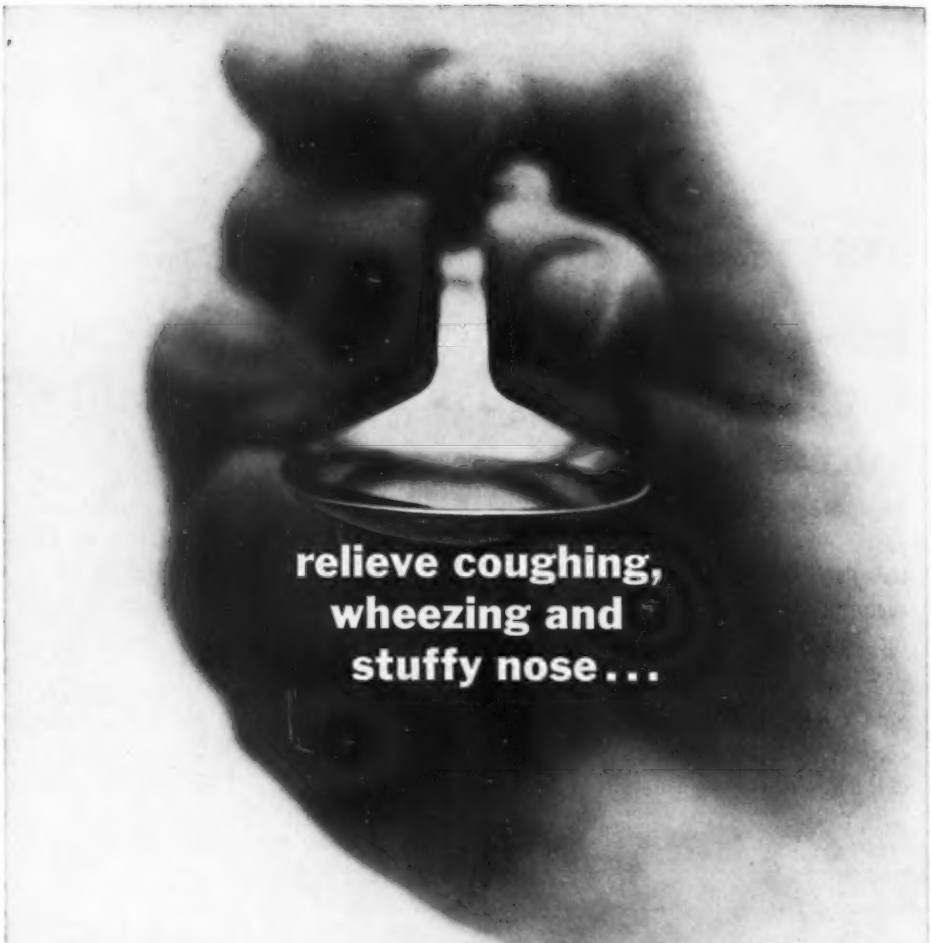
NEW, IMPROVED VI-SOL® VITAMINS

	Tri-Vi-Sol®		Poly-Vi-Sol®†		Deca-Vi-Sol®†	
	Drops (per 0.6 cc.)	Chewable Vitamins (per tablet)	Drops (per 0.6 cc.)	Chewable Vitamins (per tablet)	Drops (per 0.6 cc.)	Chewable Vitamins (per tablet)
Vitamin C (mg.)	60	75	60	75	60	75
Vitamin D (units)	400	400	400	400	400	400
Vitamin A (units)	3,000	4,000	3,000	4,000	3,000	4,000
Thiamine (mg.)			1	1.2	1	1.2
Riboflavin (mg.)			1.2	1.5	1.2	1.5
Niacinamide (mg.)			8	15	8	15
Pyridoxine (mg.)					1	1.2
Panthenol (mg.)					3	5
B_{12} (mcg.)					1	3
Biotin (mcg.)					30	40

†Also available in teaspoon dosage

Tri-Vi-Sol vitamin drops and Deca-Vi-Sol chewable vitamins are now also available with Iron. 84741

*J.A.M.A. *169*:41-45 (Jan. 3) 1959.



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'ACTIFED-C' brand EXPECTORANT

ANTITUSSIVE · EXPECTORANT · BRONCHODILATOR · DECONGESTANT · ANTIHISTAMINIC

The etiology of cough is such that drug therapy designed to produce relief may be called upon to provide several therapeutic actions simultaneously. The ingredients of 'Actifed-C' Expectorant were selected because they produce desirable antitussive, expectorant, bronchodilator, decongestant and antihistaminic effects.

Each 5 cc. teaspoonful contains:

'Actidil'® brand Triprolidine Hydrochloride	2 mg.
'Sudafed'® brand Pseudoephedrine Hydrochloride	30 mg.
Codeine Phosphate	10 mg.
Glyceryl Guaiacolate	100 mg.

Dosage: Adults and children over 12 years—2 tsp., 4 times daily. Children 6 to 12 years—1 tsp., 4 times daily. Infants and children up to 6 years—½ tsp., 4 times daily.

Precaution: Although pseudoephedrine hydrochloride causes virtually no pressor effect in normotensive patients, it should be used with caution in patients with hypertension. In addition, even though triprolidine hydrochloride produces only a low incidence of drowsiness, appropriate precautions should be observed.

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FOR EFFICIENT TOPICAL MANAGEMENT OF ECZEMATOID DERMATITIS AND OTHER SKIN DISORDERS

new methatar Creme

amino acid/antiseptic
with Liquor Carbonis Detergens

"objectively [in a series of 13 patients]* there was little difference between the hydrocortisone cream and Methatar"

Severe infectious eczematoid dermatitis, recurrent over one year

Improvement after 4 days' treatment with (L) hydrocortisone and (R) METHATAR

Severe chronic hand eczema of one year's duration

Improvement after 10 days' treatment with (L) hydrocortisone and (R) METHATAR

*Wise, L. J., Jr., and Derbes, V. J.: Evaluation of Methatar in eczema, South. M. J. 54:1031, Sept., 1961.

EXTENDING THE "metha" BENEFITS TO MORE PATIENTS IN A WIDER AREA OF DERMATOLOGIC USAGE

new methatar* Creme

Composition: An amino acid/antiseptic formulation consisting of a protein hydrolysate, methionine, benzethonium chloride, and Liquor Carbonis Detergens.

relieves itching and burning and promotes healing through the tissue-regenerative action of a protein hydrolysate reinforced by the amino acid, methionine

guards against secondary infection due to pyogenic staphylococci and other organisms by providing the antiseptic, germicidal effect of benzethonium chloride

helps establish a physiologic epidermal pH through the buffering action of the protein hydrolysate

affords the soothing relief of an emollient, water-washable vehicle (DERMABASE*)— nonstaining and greaseless

provides complementary control of itching and curbs inflammation and exudation through the antipruritic, anti-inflammatory, and drying action of the coal-tar derivative, Liquor Carbonis Detergens

extends the benefits of topical amino acid/antiseptic therapy to patients of all ages — in the youngest to the oldest

Note: Available on request — The Borden Company's Concise Guide to Treatment of Skin Disorders with "METHA" Topicals

AND TO PROVIDE FLEXIBILITY OF CHOICE FOR INDIVIDUALIZED TREATMENT

new methaphor* Ointment

Composition: An amino acid/antiseptic formulation consisting of a protein hydrolysate, methionine, camphor, and benzethonium chloride.

for minor skin disorders — affords prompt symptomatic relief, rapid healing, and protection against secondary infection

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Composition: An amino acid/antiseptic formulation consisting of methionine and benzethonium chloride, together with zinc lactate, as a mild astringent and antiseptic.

for use as a wet dressing or soak in acute dermatitis, and in exudative and chronic eczema

Supplied:
METHATAR Creme — 1½ oz. tubes
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METHASEPTIC Powder — Package of 12 envelopes

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Borden's PHARMACEUTICAL DIVISION
350 Madison Avenue
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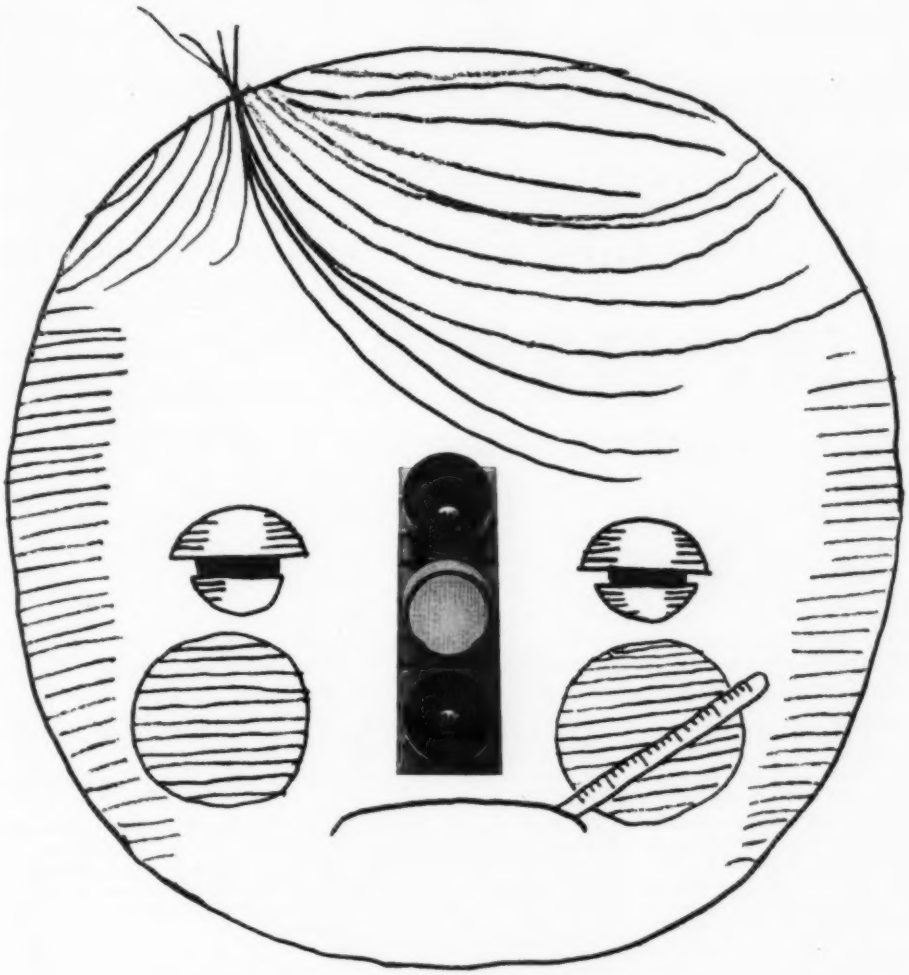
yellow for caution congested nose—secretion thick and yellow—a sign of secondary bacterial invasion—a signal for TRISULFAMINIC. A prescription for Trisulfaminic provides Triaminic® for congestion and to promote drainage of nasal and paranasal passages, and triple sulfas to provide control of streptococcal, pneumococcal and staphylococcal invaders.

Trisulfaminic®

TRIAMINIC WITH TRIPLE SULFAS TABLETS/SUSPENSION



Each new, convenient, small-size Trisulfaminic Tablet and each tsp. (5 ml.) of Trisulfaminic Suspension provides: Triaminic® 25 mg. (phenylpropanolamine hydrochloride 12.5 mg., pheniramine maleate 6.25 mg., pyrilamine maleate 6.25 mg.) and Trisulfapyrimidines, U.S.P. 0.5 Gm. **Dosage: Adults**—2 to 4 tablets or tsp. initially, followed by 2 every 4 to 6 hours. **Children 8 to 12**—2 tablets or tsp. initially, followed by 1 every 6 hours. **Children under 8**—Initially, ½ tsp. per 10 lbs. body weight, to a maximum dose of 2 tsp., then about ⅓ of this dose every 6 hours. Medication should be continued until patient has been afebrile for 3 days.

DORSEY LABORATORIES • *a division of The Wander Company* • Lincoln, Nebraska

new...
prolonged
antipruritic action
in a pleasant-tasting
chewable tablet

tacaryl®

chewable tablets

METHDILAZINE, MEAD JOHNSON

prolonged antipruritic / antiallergic action...
not dependent on delayed intestinal release

Itching in children can now be controlled on b.i.d. dosage with a long-acting[1] antipruritic/antiallergic chewable tablet your pediatric patients will enjoy taking. They can also benefit by the effectiveness of Tacaryl Hydrochloride in controlling symptoms in a wide variety of allergic conditions,[2-8] including hay fever and perennial rhinitis.

dosage: One Chewable Tablet (3.6 mg.) twice daily. Adjustment of dose or interval may be desirable for some patients.

contraindications: There are no known contraindications.

side effects: Drowsiness has been observed in a small percentage of patients. Dizziness, nausea, headache, and dryness of mucous membranes have been reported infrequently.

cautions: If drowsiness occurs after administration of Tacaryl Chewable Tablets or Tacaryl Hydrochloride, the patient should not drive a motor vehicle or operate dangerous machinery. Since Tacaryl Chewable Tablets or Tacaryl Hydrochloride may display potentiating properties, it should be used with caution for patients receiving alcohol, analgesics or sedatives (particularly barbiturates). Because of reports that phenothiazine derivatives occasionally cause side reactions such as agranulocytosis, jaundice and orthostatic hypotension, the physician should be alert to their possible occurrence... though no such reactions have been observed with Tacaryl Chewable Tablets or Tacaryl Hydrochloride.

supplied: Pink tablets, 3.6 mg., bottles of 100.

references: (1) Lish, P. M.; Albert, J. R.; Peters, E. L., and Allen, L. E.: Arch. internat. pharmacodyn. *129*:77-107 (Dec.) 1960. (2) Howell, C. M., Jr.: North Carolina M. J. *21*:194-195 (May) 1960. (3) Clinical Research Division, Mead Johnson & Company. (4) Wahner, H. W., and Peters, G. A.: Proc. Staff Meet. Mayo Clin. *35*:161-169 (March 30) 1960. (5) Crepea, S. B.: J. Allergy *31*:285-285 (May-June) 1960. (6) Crawford, L. V., and Grogan, F. T.: J. Tennessee M. A. *53*:307-310 (July) 1960. (7) Spoto, A. P., Jr., and Sieker, H. O.: Ann. Allergy *18*:761-764 (July) 1960. (8) Arbesman, C. E., and Ehrenreich, R.: New York J. Med. *61*:219-229 (Jan. 15) 1961.

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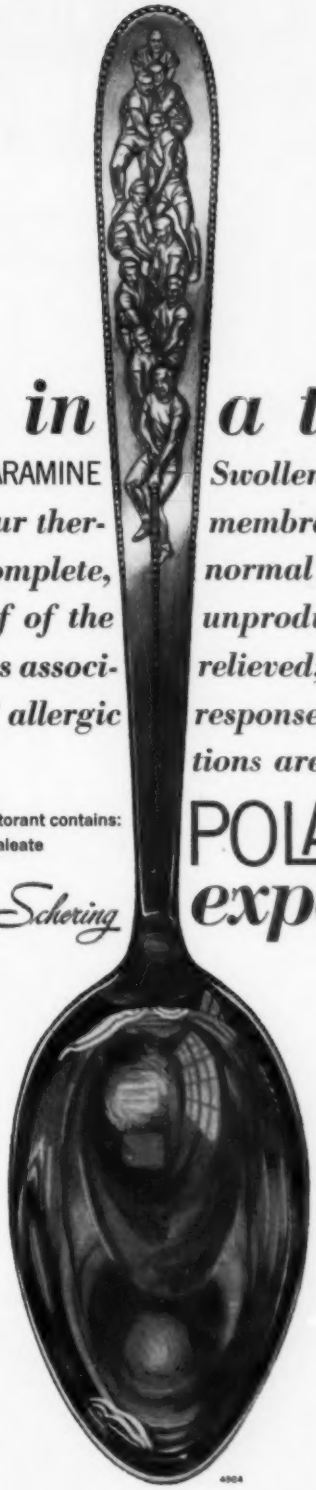
MARCH, 61

teamwork in a teaspoon



Each teaspoonful of POLARAMINE ***Expectorant provides four therapeutic effects for more complete, rapid and effective relief of the coughs and complications associated with your patients' allergic respiratory disorders.***

Swollen, congested mucous membranes are returned to normal rapidly, gently; dry, unproductive coughing is relieved; and further allergic response and its manifestations are reduced.

Each 5 cc. teaspoonful of POLARAMINE Expectorant contains:
2 mg. POLARAMINE (dexchlorpheniramine) Maleate
20 mg. d-Isoephedrine Sulfate
100 mg. Glyceryl Guaiacolate
Supply: 16 oz. bottles.

Schering

POLARAMINE®
expectorant

vitamin K_1 and the disappearance of neonatal hemorrhage

"The widespread use of vitamin K in the newborn has changed what was once a major pediatric problem . . . into a matter of only academic interest."*

KONAKION provides the most efficient means of routine vitamin K prophylaxis: faster rate of absorption than menadione or its derivatives, wider margin of safety than vitamin K analogs; both oral and parenteral dosages (the parenteral form is a fine aqueous dispersion compatible with most intravenous vehicles). Not a single case of kernicterus due to KONAKION has been reported. KONAKION is supplied in low-dosage ampuls, permitting economical one-time use.

* A. I. Aballi, V. L. Banus, S. de Lamerens and S. Rozengvaig, J. Dis. Child. 97:524, 1959. And abstr. in J.A.M.A., 170:2249, 1959 and Nutrition Rev. 17:229, 1959.

Capsules — 5 mg. Ampuls — 1 mg/0.5 cc, 10 mg/1.0 cc, 25 mg/2.5 cc

KONAKION

brand of vitamin K_1

STOP BLEEDING . . . SAFELY

ROCHE LABORATORIES
Division of Hoffmann-La Roche Inc. • Nutley 10, New Jersey

they made mine

Soyalac

Fibre-free HYPOALLERGENIC formula

1. Provides balanced nutritional values.
2. An excellent formula for regular infant feeding.
3. An ideal food for milk allergies, eczema and problem feeding.

SOYALAC helps solve the feeding problem of prematures and infants requiring milk-free diet.

Strikingly similar to mother's milk in composition and ease of assimilation, babies thrive on SOYALAC.

Clinical data furnish evidence of SOYALAC'S value in promoting growth and development.

Protein of high biologic value is obtained from the soybean by an exclusive process.

Free Booklet and Samples

A request on your professional letterhead or prescription form will bring to you complete information, and a supply of samples.

Medical Products Division

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Equilibrium for the epileptic

Better control of seizures, plus the emotional support of "physician-educated" parents, can greatly aid the child with epilepsy to prepare for normal social relationships—before she starts school.

Mebaral offers highly effective control of most types of epilepsy, especially major motor seizures in children. It reduces the number and severity of seizures—without producing sedative daze or lowering learning capacity.

Mebaral is unsurpassed in safety. It is one of the best tolerated and "...least upsetting of all forms of therapy"[1] for epilepsy. Even when used repeatedly year after year, Mebaral rarely produces toxic reactions or ill effects.

Mebroin®, a synergistic combination of Mebaral and diphenylhydantoin, provides maximal control of seizures with minimal toxicity. Side effects are infrequent. Each relatively tasteless tablet combines 90 mg. of Mebaral with 60 mg. of diphenylhydantoin.

1. Robertson, E. G.: *Postgrad. Med.* 25:31, Jan., 1959.

For epilepsy at any age

Mebaral®

Brand of mephobarbital

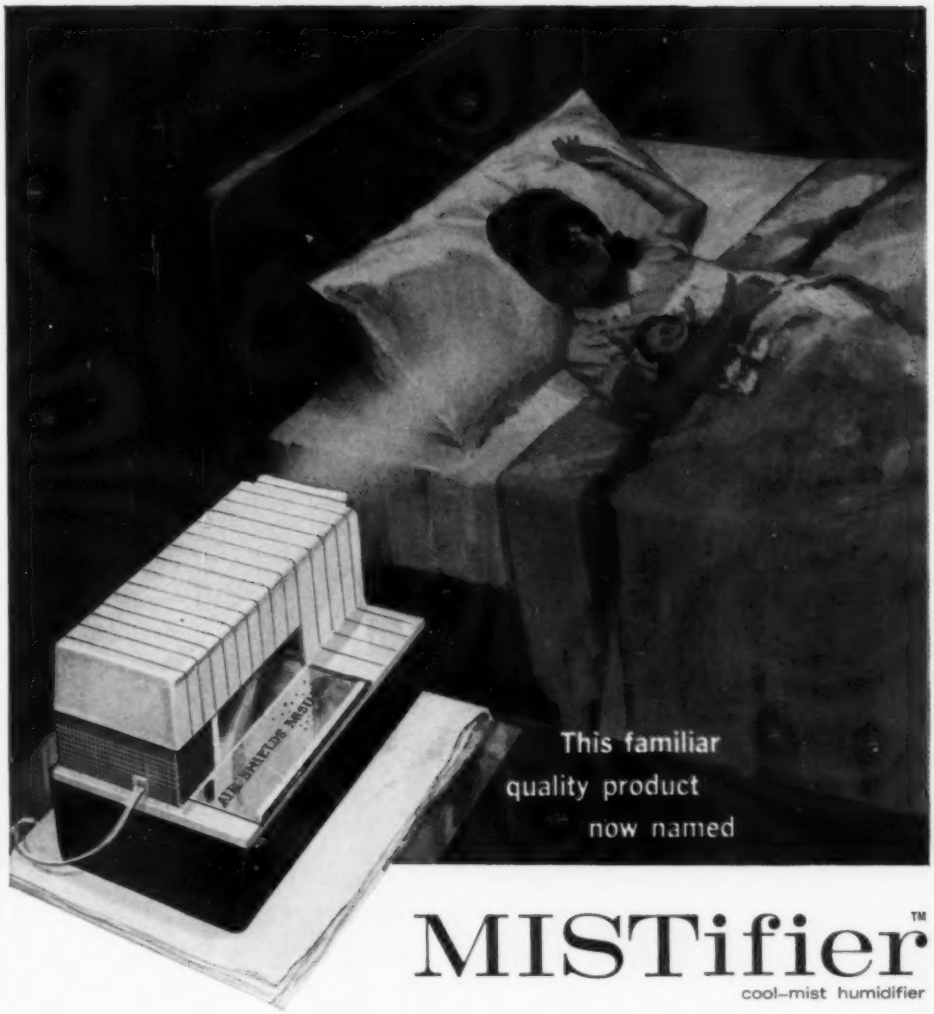
Mebaral dosage: Children under 5 years, from 16 to 32 mg. (¼ to ½ grain) three or four times daily; over 5 years, from 32 to 64 mg. (½ to 1 grain) three or four times daily. Adults, from 400 to 600 mg. (6 to 9 grains) daily.

Mebroin dosage: Children under 6 years, ½ tablet once or twice daily; over 6 years, 1 tablet two or three times daily. Adults, 1 or 2 tablets three times daily (average dosage).

How Supplied: Mebaral tasteless tablets of 200 mg. (3 grains), 100 mg. (1½ grains), 50 mg. (¾ grain), and 32 mg. (½ grain), bottles of 100. Mebroin virtually tasteless tablets, bottles of 100 tablets.

Winthrop LABORATORIES
New York 18, N. Y.

Before prescribing be sure to consult Winthrop's literature for additional information about dosage, possible side effects and contraindications.



MISTifier™

cool-mist humidifier

FOR DIRECT INHALATION

- Directional "Fog Stream" of cool vapor
- Hydrates respiratory tract
- Loosens secretions
- No heat—no tent

Operates for 10 hours on one filling.

Used as a room humidifier the MISTifier combats dehydration of the respiratory tract due to lack of moisture in hospital or bedroom.

Now available through leading pharmacies and surgical supply houses.

Write for the name of your nearest distributor.

AIR-SHIELDS, INC.

Hatboro, Pennsylvania

A Division of **National Aeronautical Corporation**

At the Child Life "LAND OF HAPPY FEET" there are four basic shoes that readily adapt to your every prescription need.

- **ARCH FEATURE**
- **STRAIGHT LAST**
- **SURGICAL BOOTS**
- **REGULAR SHOES**

Plus Mismate Service from factory stock. Specially trained shoe fitters will accurately follow your prescription — with shoe styles your patients will enjoy wearing.

Child Life
SHOES

MERIT YOUR PROFESSIONAL RECOMMENDATION

HERBST SHOE MANUFACTURING CO., Box 2005, Milwaukee 1, Wisconsin

the iron you can depend on

PEDIATRIC PRODUCTS
KINNEY & COMPANY, INC.
Columbus, Indiana

for continued effectiveness

- *clinical effectiveness confirmed*[1]
- *palatable–free from astringency*
- *well tolerated even between meals*
- *easily mixed with formula, milk, juices*
- *does not stain teeth*

the drop dose form

CHĒL-IRON®

Brand of Ferrocholinate*

PEDIATRIC DROPS

(25 mg. iron/cc.)

the teaspoon dose form

CHĒL-IRON®

Brand of Ferrocholinate

LIQUID

(50 mg. iron/tsp.)

because it's chelated Chēl-Iron offers optimal assurance of effective therapy for more of your patients. Because it is neither ionized nor precipitated after ingestion, Chēl-Iron rarely causes the gastrointestinal complaints often experienced with nonchelated iron salts, such as ferrous sulfate or ferrous gluconate.[1,2] Thus, your supplemental or therapeutic iron regimen is uninterrupted, and full hematologic benefits are *maintained*.

1. Franklin, M., et al.: J.A.M.A. 166:1685, 1958. 2. A.M.A. Council on Drugs: New and Nonofficial Drugs 1960, Philadelphia, Lippincott, 1960, p. 521.

*U.S. Pat. 2,575,611

PROMPTLY ANSWERS THE CALL

for relief of nausea and vomiting

EMETROL®

Phosphorated Carbohydrate Solution

Make your first thought EMETROL—whenever an antiemetic is indicated, as in acute infectious gastroenteritis or intestinal "flu," and in the prevention or treatment of nausea due to drug therapy or motion sickness.

EMETROL quickly controls most cases of functional nausea and vomiting without risk of untoward effects or masking of serious organic pathology.

Supplied: Bottles of 3 fl.oz. and 16 fl.oz. through all pharmacies.

EMETROL Bibliography: 1. Bradley, J. E., et al.: J. Pediat. 38:41, 1951. 2. Proutt, L. M., et al.: Bull. School Med. Univ. Maryland 36:86, 1951. 3. Levenstein, I.: Report of Leberco Laboratories, Roselle Park, N. J. 4. Bradley, J. E.: Mod. Med. 20:71, No. 20, 1952. 5. Tebrock, H. E., and Fisher, M. M.: M. Times 82:271, 1954.

in clinical use for 10 years... not a single report of side effects

Kinney

PEDIATRIC PRODUCTS

KINNEY & COMPANY, INC. Columbus, Indiana

Thousands of doctors answer this question by telling mothers to give their children St. Joseph Aspirin For Children

St. Joseph Aspirin For Children a medication of choice whenever salicylate therapy is indicated in infancy and childhood

Doctors trust St. Joseph Aspirin For Children. They trust it because for the past 12 years it has proved itself in every way an excellent aspirin tablet for children. It has become a preferred aspirin in the pediatrician's armamentarium. One reason for this preference is because Plough pioneered this specialized aspirin tablet for children. Another reason is that each tablet contains 1¼ grains of highest quality aspirin—the dosage most widely approved by doctors. Still another reason is because St. Joseph Aspirin For Children is an elegant pharmaceutical of exceptional palatability.

Where a matter of taste makes a world of difference

There's never a need to worry about any fussing, fretting, high temper or frustrating tension when a mother gives her child St. Joseph Aspirin For Children. That's because this specialized 1¼ grain aspirin tablet has a pleasant orange flavor, and a smooth, creamy texture.

Try it. Put a tablet of St. Joseph Aspirin For Children on your tongue. Let it dissolve. Note how pleasant it tastes. Then do the same with any other children's aspirin tablet and you'll discover why so many mothers prefer giving St. Joseph Aspirin For Children—why children accept it readily.

St. Joseph Aspirin For Children pioneered the safety cap for children's aspirin

Yes! It was St. Joseph Aspirin For Children that first provided children's aspirin with a truly effective Safety Cap. This is the now famous plastic cap that cannot be twisted off. Pressures against the sides of the cap make it hold even tighter. The cap can be removed only by pressure applied at just the right point—through the narrow vertical groove in the rim of the bottle. The part played by Plough, Inc., in developing this Safety Cap has been widely recognized by the medical profession.

If you would like to have samples of St. Joseph Aspirin For Children for your patients, write directly to Plough, Inc., Memphis, Tennessee.

In Canada,
write for samples
of ST. JOSEPH BEBETINE
FOR CHILDREN.

Plough, Inc.

New York • Memphis • Los Angeles • Miami

*the decontussive** =

DOSAGE (to be administered every 3 or 4 hours): Children 6 to 12 – 1 tsp.; 1 to 6 – ½ tsp.; under 1 – ¼ tsp.; Patients over 12 – 2 tsp. One dose at bedtime is usually sufficient to control the cough cycle initiated by postural drainage of paranasal sinuses.

better cough control

Triaminicol®

*the decongestant-antitussive cough syrup

reduces postnasal drainage ■ lessens pharyngeal irritation
depresses the cough reflex ■ eases expulsion of mucus

Irritation caused by postnasal drip is a particularly important factor in pediatric cough. Adding the outstanding decongestant action of Triaminic® to the central antitussive action of Dormethan® and expectorant action of ammonium chloride provides cough control distinctly superior to that afforded by ordinary non-decongestant cough syrup.

Each tsp. (5 ml.) of fruit-flavored, non-alcoholic TRIAMINICOL provides: Triaminic® 25 mg. (phenylpropanolamine HCl 12.5 mg., phenyramine maleate 6.25 mg., pyrilamine maleate 6.25 mg.); Dormethan® (brand of dextromethorphan HBr) 15 mg.; Ammonium chloride 90 mg.

DORSEY LABORATORIES · *a division of The Wander Company* · Lincoln, Nebraska

Why meat animals are modernized

New concepts in human nutrition must be met, whatever they are!

A few years ago everyone seemed to prefer fat type, well marbled meat. Today, the demand is for less fat, more lean meat.

To change animals is not easy.. and it has required years of scientific development. Through careful breeding, specialized feeding and continuing research, livestock men now are raising more and more lean-meat animals... modern animals!

"Just what the doctor ordered."

Through further research and modernized methods, such as, tenderizing and closer trim, processors cooperate to bring to retail stores the flavorful, appetizing meat on display everywhere... so attractive to all.

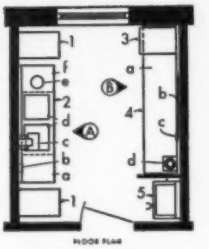
MEAT–*a universally preferred source of*
HIGH QUALITY PROTEIN

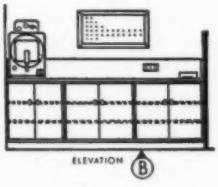
AMERICAN | ***MEAT*** | ***INSTITUTE***

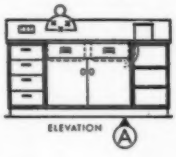
MAIN OFFICE, CHICAGO • MEMBERS THROUGHOUT THE NATION

HERE!------

a low cost practical technic for the small INFANT-FORMULA ROOM







The Amsco electric heat Model 832 Formula Sterilizer enables hospitals with one to eight bassinets to carry out advanced techniques formerly possible only with large capacity equipment costing much more. Its capacity of 32 four or eight ounce bottles is ample to serve eight bassinets, using two cycles per day. The Model 832 requires minimum attention from the operator because complete cycling is automatic and forgetproof according to the time, temperature and exhaust settings selected.

Permits a Modern Formula Room in the minimum area.

With the model 832 as the basic unit, Amsco has developed special techniques and complete equipment suitable to Formula Room planning for the small nursery. Layout and equipment are fully in harmony with the most advanced standards of infant formula processing and work simplification . . . yet the space requirements and all-inclusive costs are extremely modest.

For complete information on the small Infant Formula Room, write for bulletin SC-319. (Hospitals with larger nurseries should request brochure SC-320R.)

World's largest Designer and Manufacturer of Surgical Sterilizers, Tables, Lights and related equipment.

AMERICAN STERILIZER
ERIE • PENNSYLVANIA

an added measure of protection for little patients

against relapse
against "problem" pathogens

DECLOMYCIN®

DEMETHYLCHLORTETRACYCLINE LEDERLE

pediatric drops
syrup

● full antibiotic activity ● lower milligram intake per dose ● up to 6 days' activity with 4 days' dosage ● uniformly high, sustained peak activity ■ **syrup** (cherry-flavored), 75 mg./5 cc. tsp., bottles of 2 and 16 fl. oz. Dosage: 3 to 6 mg./lb./day—in four divided doses. **pediatric drops,** 60 mg./cc., 3 mg./drop, 10 cc. bottles with calibrated dropper. Dosage: 1 to 2 drops/lb./day—in four divided doses

PRECAUTIONS: As with many other antibiotics, DECLOMYCIN may occasionally give rise to glossitis, stomatitis, proctitis, nausea, diarrhea, vaginitis or dermatitis. A photodynamic reaction to sunlight has been observed in a few patients on DECLOMYCIN. Although reversible by discontinuing therapy, patients should avoid exposure to intense sunlight. If adverse reaction or idiosyncrasy occurs discontinue medication. Overgrowth of nonsusceptible organisms is a possibility with DECLOMYCIN, as with other antibiotics. The patient should be kept under observation.

Request complete information on indications, dosage, precautions and contraindications from your Lederle representative, or write to Medical Advisory Department.

LEDERLE LABORATORIES, a Division of AMERICAN CYANAMID COMPANY, Pearl River, New York

WHY ACID MANTLE?

A DISTINCT THERAPEUTIC ENTITY*
Restores and maintains skin's normal protective acidity—speeds natural healing and helps sensitive skin resist irritants and infection.

A NOTABLE VEHICLE
Special water-miscible, evaporable base assures better dispersion, greater concentration of active ingredients in contact with skin—increases response through its own therapeutic action.

*Supplied: in Creme and Lotion (pH 4.6)

IN THE TREATMENT OF STUBBORN DERMATOSES, YOU CAN EXPECT RESULTS LIKE THESE WITH COR-TAR-QUIN®

pH 5.0

Response of 113 Patients with various skin disorders to Cor-Tar-Quin Creme and Lotion†

Condition	No. of Cases	Complete Remission	Improved	No Response
Seborrheic dermatitis	37	31	6	0
Neurodermatitis	41	18	22	1
Atopic eczema	16	7	9	0
Tinea cruris	3	1	1	1
Other dermatoses	16	4	12	0
TOTALS	113	61 (54%)	50 (44%)	2 (2%)

†Adapted from Olansky, S.

"Especially effective"[1] for lesions characterized by SCALING, LICHENIFICATION AND INFECTION

Description: Cor-Tar-Quin is a unique topical creme or lotion, combining micro-dispersed hydrocortisone alcohol, ½%, or liquor carbonis detergens 2% and diiodohydroxyquinoline 1% in the Acid Mantle® vehicle.

Reference: (1) Olansky, S.: Antimicrobial-Steroid-Tar Combination in Treatment of Subacute and Chronic Dermatoses, J.M.A. Georgia **50**:398 (Aug.) 1961.

02661

WORLD LEADER IN DERMATOLOGICALS
DOME CHEMICALS INC.
NEW YORK 23, NEW YORK

A CHILD'S PAIN IS NOT AN ADULT'S PAIN
cut
down
to
size

a new product specifically synthesized for children

analexin® syrup

Powerful Analgesia with Antipyretic Response

pain is pain... it hurts!

the difference is that in children, pain is more frequently accompanied by fever.

New Analexin Syrup's analgesic effectiveness is equal to virtually any pain . . . even the severe pain of otitis or that of injury or trauma. Its inherent antipyretic response combats associated fever.

Analexin Syrup also has an unparalleled margin of safety. It is non-narcotic and not narcotic related. There is no evidence suggestive of tolerance or cumulative toxicity. No adverse effects have been reported on bone marrow, cardiovascular, respiratory, kidney or central nervous systems.

FORMULA:

Each 5 cc. contains 100 mg. of phenyramidol salicylate.

DOSAGE:

Children under 3 years old, 1 teaspoonful 3 or 4 times daily; 3 to 12 years old, 2 teaspoonfuls 3 or 4 times daily.

ANALEXIN SYRUP IS ANOTHER ACHIEVEMENT OF NEISLER RESEARCH

IRWIN, NEISLER & CO. DECATUR, ILLINOIS

MAIL THIS CARD TODAY FOR A COMPLIMENTARY TRIAL SUPPLY OF ANALEXIN SYRUP AND SUCH OTHER NEISLER PEDIATRIC MEDICATION AS MAY INTEREST YOU. (SEE REVERSE SIDE OF THIS PAGE)

PLEASE SEND ME A TRIAL SUPPLY OF:

analexin® syrup ☐

PLEASE LIST ANY OTHER PRODUCTS OF WHICH YOU WOULD LIKE SAMPLES:

_____

_____

_____

NAME_____
(PLEASE PRINT)

ADDRESS_____

CITY_____ ZONE_____ STATE_____

AUTHORIZED SIGNATURE_____
(THIS CARD MUST BE SIGNED BY A LICENSED PHYSICIAN.)

J

why formulate products specifically for pediatric use?

Many pediatric preparations are merely fractional doses of products for adults. These "cut down versions" often neglect the specialized therapy required in pediatrics. Of primary importance are precisely adjusted dosages that will be as well tolerated by children as they are therapeutically effective. Such care is rewarded by the superior results it produces. *This is the rationale behind Neisler pediatric products.*

But production is only half the task. The end product must be proven both for efficacy and safety. Again, Neisler takes special steps by having its pediatric products clinically tested by pediatricians. For example, Rynatan and Rynatuss Suspension formulations have more clinical papers[1-9] attesting to the therapeutic efficacy of their ingredients than any other products for the same indications.[1-4] In addition, these two medications are the only ones of their type to contain DURABOND®, a sustained action principle whose performance has been definitely established, by radioactive tracer studies, to control drug blood levels in humans.[5]

That your satisfaction—and that of your patients and their parents—is assured before you write your first prescription for any of the Neisler pediatric products listed below:

RYNATAN® Suspension—for long-acting nasal decongestion

FORMULA: Each 5 cc. contains pyrilamine tannate, 12.5 mg.; phenylephrine tannate, 5.0 mg.; chlorpheniramine tannate, 2.0 mg.

DOSAGE: Each 12 hours: Children 6 mos.-2 yrs., 1/2 tsp.; 2-6 yrs., 1/2-1 tsp.; 6-12 yrs., 1-2 tsp.

RYNATUSS® Suspension—for long-acting cough control and decongestion

FORMULA: Each 5 cc. contains carbetapentane tannate (non-narcotic), 40 mg.; chlorpheniramine tannate, 4 mg.; ephedrine tannate, 5 mg.; phenylephrine tannate, 5 mg.

DOSAGE: Each 12 hours: Children 6 mos.-2 yrs., 1/2 tsp.; 2-6 yrs., 1/2-1 tsp.; 6-11 yrs., 1-2 tsp.

DAINITE® Pediatric Tablets—expressly for the asthmatic child

Dainite Pediatric tablets relieve bronchoconstriction, produce a marked increase in vital capacity, with minimum risk of side effects. An exclusive feature, protected aminophylline (U. S. Pat. No. 566,376), minimizes the risk of gastric intolerance and thus insures sustained therapeutic aminophylline blood levels.[10-14]

FORMULA: Each tablet contains: aminophylline, 1 gr.; ephedrine HCl, 1/12 gr.; phenacetin, 1/8 gr.; ethyl aminobenzoate, 1/8 gr.; aluminum hydroxide, 1/2 gr.

DOSAGE: Age 6-6 yrs., 1 tablet at 8 a.m., 4 p.m. and at bedtime. 6-12 yrs., 2 tablets at the above times. Or as directed by the physician.

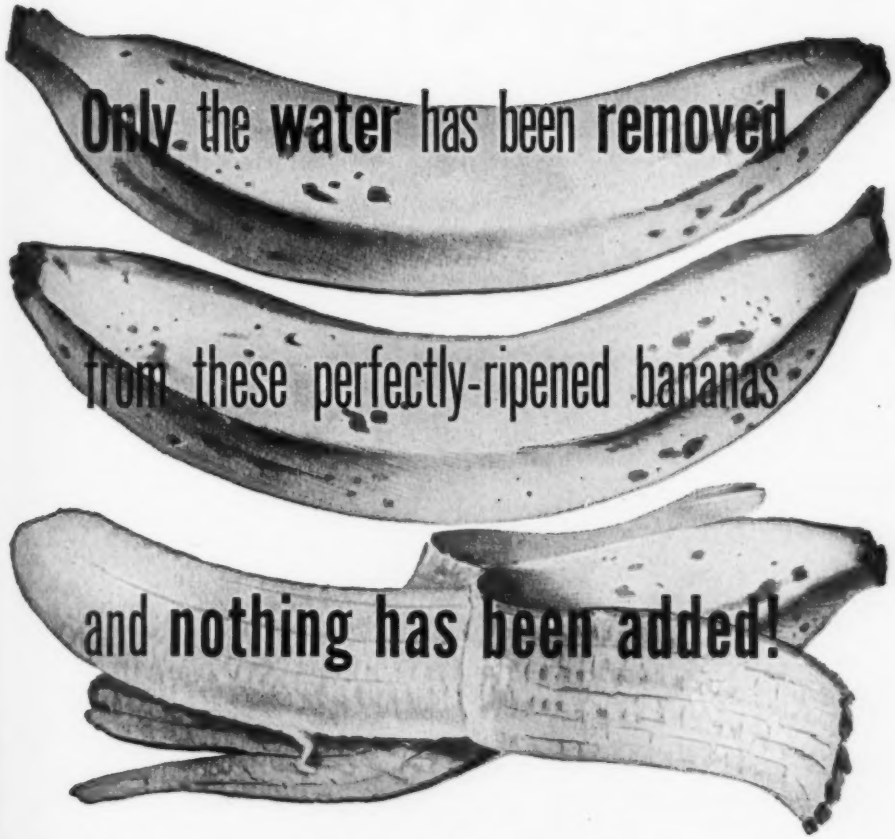
1. Research and Clinical Observations Favorable to Rynatan and Rynatuss. Medical Digest, 4:4, Dec. 1956. 2. Hamilton, R. E., Jr., Dr. Med. E. (A.), and Schwartz, F. D., Southern Med. J., 49:6, 1956. 3. Wilson, L., and Wilson, R. B., J.A.M.A., Vol. 161, No. 6, 1956. 4. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956. 5. Wilson, L., and Schwartz, L., New York J. Med., 57:1956. 6. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956. 7. Wilson, L., and Schwartz, L., New York J. Med., 57:1956. 8. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956. 9. Wilson, L., and Schwartz, L., New York J. Med., 57:1956. 10. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956. 11. Wilson, L., and Schwartz, L., New York J. Med., 57:1956. 12. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956. 13. Wilson, L., and Schwartz, L., New York J. Med., 57:1956. 14. Lovell, R. R., and Chapman, W. Chr., Ohio State J. Clin. Med., 7:10, 1956.

FIRST CLASS
PERMIT NO. 6,
DECATUR,
ILLINOIS

BUSINESS REPLY MAIL
NO POSTAGE NECESSARY IF MAILED IN THE UNITED STATES

POSTAGE WILL BE PAID BY

Pediatric Service
Medical Department
IRWIN, NEISLER & CO.
P. O. Box 1110
Decatur, Illinois



Kanana Banana Flakes for *instant* mashed banana in daily routine feeding!

• ***PURE*** • ***PERFECTLY RIPE***
• ***FULLY NUTRITIOUS***

The addition of three parts water or milk will INSTANTLY provide the wholly-nutritious mashed banana you prescribe.

When you prescribe banana, write "Kanana"!

Kanana Banana Flakes (NOT A POWDER)

FREE SAMPLE! Fill out and mail TODAY! For content analysis and free sample, send to:

Kannengiesser & Co., 76 Ninth Ave., New York 11, N. Y.

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KANNENGIESSER & COMPANY, 76 NINTH AVE., NEW YORK 11, NEW YORK. In Canada: Laurentian Agencies, Ltd., 429 St. Jean Baptiste Street, Montreal, Canada

The taste says, Yes!

BICILLIN®

ORAL SUSPENSION

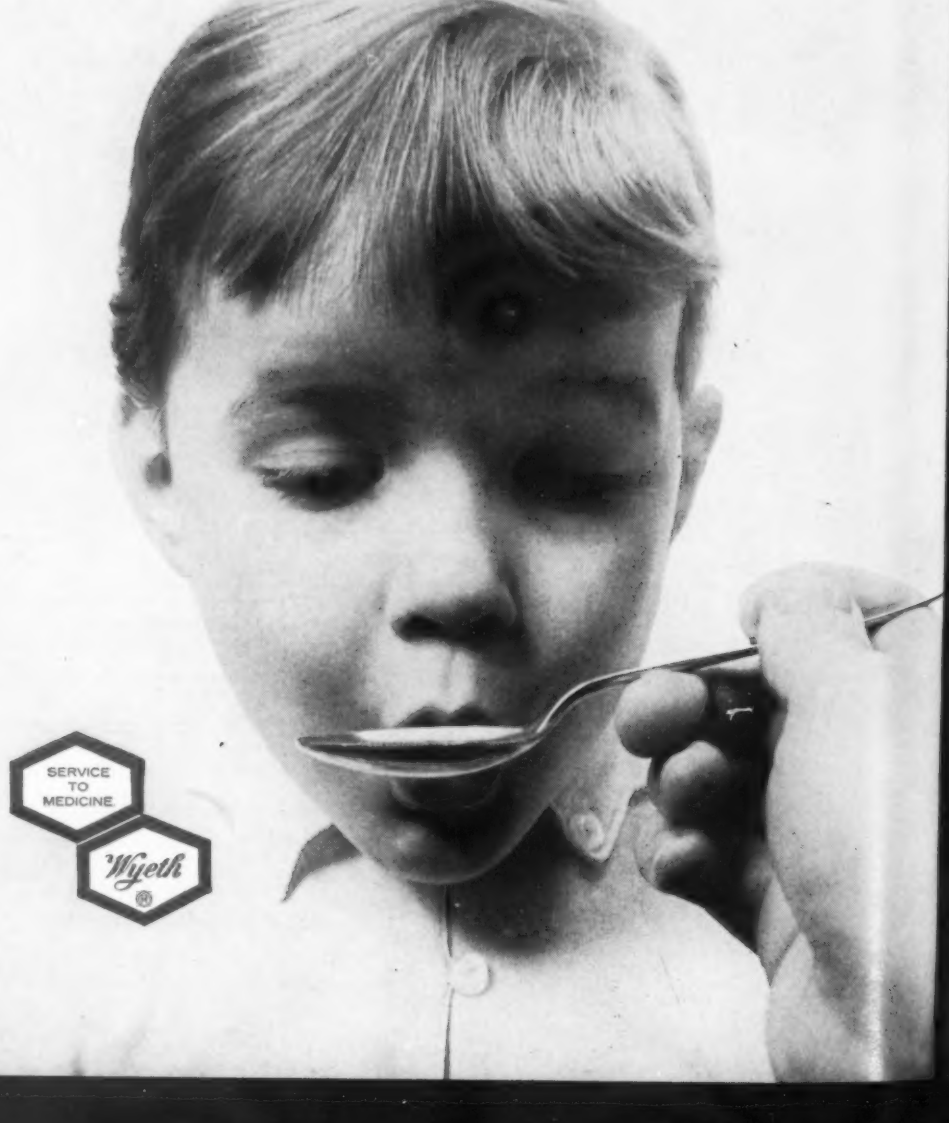
STABLE!
READY TO USE!

Benzathine Penicillin G, Wyeth (Dibenzylethylenediamine Dipenicillin G)

A Superior Oral Penicillin for Children

SUPPLIED: *Cherry flavor*—300,000 units per 5-cc. tsp., bottles of 2 fl. oz.
Custard flavor—150,000 units per 5-cc. tsp., bottles of 2 fl. oz.

Wyeth Laboratories, Philadelphia 1, Pa.



SERVICE TO MEDICINE

Wyeth®

color, aroma, taste.....all orange!!

PALADAC®

Liquid Vitamin Preparation

The three oranges of PALADAC make it a triple favorite with children because they love its lively orange color...its fragrant orange aroma...and its delicious orange flavor. A well-balanced nine vitamin formula, PALADAC is valuable for prevention of vitamin deficiencies. It may be given directly or incorporated into milk, fruit juice, or practically any other food. And PALADAC is stable...requires no refrigeration. *available:* 4-ounce and 16-ounce bottles.

62661

PARKE-DAVIS

PARKE, DAVIS & COMPANY, Detroit 32, Michigan

today's exposure...

Whatever the cause—there is no safer way to clear little heads and provide day-long (or night-long) relief of congestion miseries than Rynatan Suspension.

Expressly for Pediatrics

RYNATAN® SUSPENSION

Each 5 cc. contains: pyrilamine tannate, 12.5 mg.; phenylephrine tannate, 5.0 mg.; chlorpheniramine tannate, 2.0 mg. **Children:** Each 12 hours—6 mos.-2 yrs., 1/3 tsp.; 2-6 yrs., 1/2-1 tsp.; 6-11 yrs., 1-2 tsp.

in Sinusitis,
Rhinitis,
other UR congestion

RYNATAN® SUSPENSION

when you prescribe Rynatan Suspension, you can be assured that it is

unsurpassed

for long-acting decongestion

No other liquid oral nasal decongestant for children contains DURABOND®, a sustained action principle whose performance has been definitely established, by radioactive tracer studies, to control drug blood levels in humans.[1]

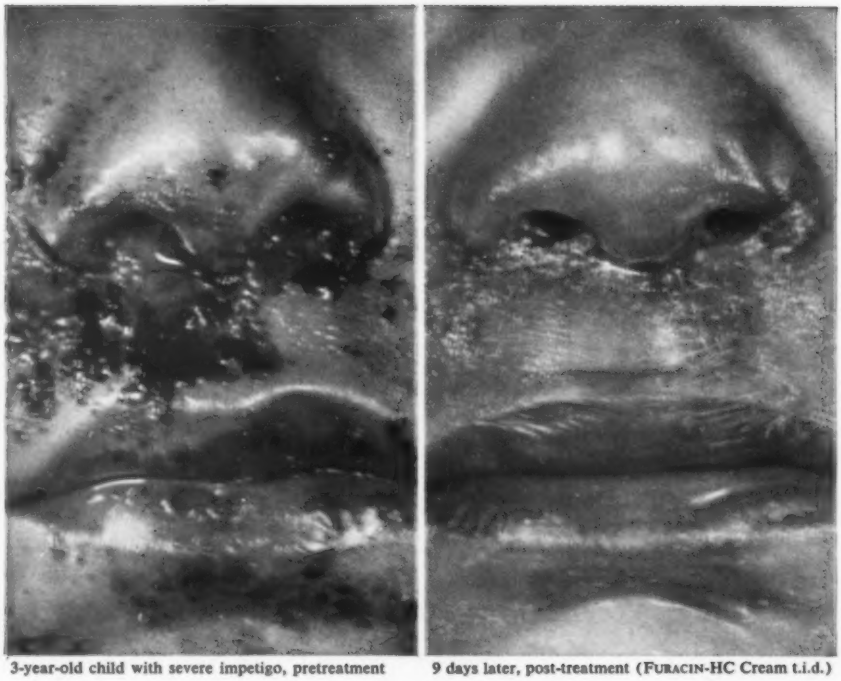
unsurpassed

in published proof of effectiveness

No other liquid oral nasal decongestant for children can provide as many published clinical papers testifying to its therapeutic efficacy as Rynatan.[2-9]

1. Bogner, R. L., and Moses, C.: Evaluation of a Sustained Release Principle in Human Subjects Utilizing Radioactive Techniques, to be published. 2. Report on a New Repository Principle, Med. Sc. *3*:376, 1958. 3. Steller, R. E.; DeMar, E. A., and Schwartz, F. R.: Indust. Med. & Surg. *28*:362, 1959. 4. Villanyi, L., and Stillwater, R. B.: E.E.N.T. Monthly *38*:650, 1959. 5. Lawler, E. G., and Limperis, N. M.: Clin. Med. *5*:1669, 1958. 6. Simon, D.: Clin. Med. *7*:1761, 1960. 7. Sherwood, H., and Epstein, J.: New York J. Med. *60*:1793, 1960. 8. Kile, R. L.: Antibiotic Med. & Clin. Therap. *5*:578, 1958. 9. Lanoff, G.: Illinois M.J. *117*:16, 1960.

IRWIN, NEISLER & CO. *75th year* DECATUR, ILLINOIS



3-year-old child with severe impetigo, pretreatment

9 days later, post-treatment (FURACIN-HC Cream t.i.d.)

FOR BROADER TREATMENT OF INFLAMMATORY SKIN DISORDERS, BOTH ACUTE AND CHRONIC, WHERE INFECTION IS PRESENT OR IMMINENT

FURACIN®-HC CREAM

nitrofurazone 0.2% and hydrocortisone acetate 1%, Eaton

ESPECIALLY USEFUL FOR THE TREATMENT OF INFLAMMATION, ERYTHEMA AND PRURITUS AS WELL AS INFECTION IN SUCH CASES AS PYODERMAS, FURUNCULOSIS AND SECONDARILY INFECTED DERMATOSES

FURACIN-HC Cream combines the anti-inflammatory and antipruritic effect of hydrocortisone with the dependable antibacterial action of FURACIN—the most widely prescribed single topical antibacterial. Exclusively for topical use, FURACIN retains undiminished potency against pathogens such as staphylococci that no longer respond adequately to other antimicrobials. FURACIN is gentle, nontoxic to regenerating tissue, speeds healing through efficient prophylaxis or prompt control of infection.

FURACIN-HC Cream is available in tubes of 5 Gm. and 20 Gm. Vanishing-cream base, water-miscible.

EATON LABORATORIES, Division of The Norwich Pharmacal Company, NORWICH, N. Y.

NEW **SPECIAL COUGH FORMULA**
for Children

Pediacof

Trademark

SOOTHING DECONGESTANT AND EXPECTORANT

Each teaspoon (5 cc.) contains:

Codeine phosphate	5.0 mg.
Neo-Synephrine® hydrochloride (brand of phenylephrine hydrochloride)	2.5 mg.
Chlorpheniramine maleate	0.75 mg.
Potassium iodide	75.0 mg.

Bright red, pleasant tasting, raspberry flavored syrup

Dosage:

Children from 6 months to 1 year, 1/4 teaspoon; 1 to 3 years, 1/2 to 1 teaspoon; 3 to 6 years, 1 to 2 teaspoons; 6 to 12 years, 2 teaspoons. Every four to six hours as needed.

How Supplied:

Bottles of 16 fl. oz.

Exempt Narcotic

Winthrop
LABORATORIES
New York 18, N. Y.

Before prescribing be sure to consult Winthrop's literature for additional information about dosage, possible side effects and contraindications.

wherever
monilial superinfection
*is a particular hazard**

Terrastatin®

OXYTETRACYCLINE WITH GLUCOSAMINE PLUS NYSTATIN

capsules and for oral suspension

the antibiotic effectiveness of
Terramycin enhanced
with antifungal activity

IN BRIEF

Terrastatin (oxytetracycline with glucosamine and nystatin) provides the established antibiotic dependability of Terramycin® plus the potent antifungal activity of nystatin, which has a significant prophylactic action against intestinal monilial overgrowth.

INDICATIONS: Effective against both gram-positive and gram-negative bacteria, rickettsiae, spirochetes, large viruses, and certain parasites (amebae, pinworms), Terrastatin is indicated in a great variety of infections due to susceptible organisms, e.g., infections of the respiratory, gastrointestinal, and genitourinary tracts, surgical and soft-tissue infections, ophthalmic and otic infections, and many others. The added protection afforded by Terrastatin against monilial superinfection is especially important for those patients who are most likely to be susceptible to the overgrowth of *Candida albicans.*

ADMINISTRATION AND DOSAGE: Adults: Dosage providing 1 Gm. of oxytetracycline daily in four divided doses is usually effective. In *severe* infections, 2-4 Gm. daily may be indicated. Infants and children: 10-20 mg. of oxytetracycline per lb. of body weight daily.

SIDE EFFECTS AND PRECAUTIONS: If superimposed infection caused by resistant staphylococci is observed, the antibiotic should be discontinued, and a therapeutic trial of other antibiotics as indicated by susceptibility testing may be initiated. Aluminum hydroxide gel has been shown to decrease antibiotic absorption and is therefore contraindicated. Glossitis and allergic reactions are rare. Nystatin is virtually nontoxic and nonsensitizing; side effects are seldom observed. There are no known contraindications to glucosamine.

SUPPLIED: *Terrastatin Capsules,* 250 mg. of oxytetracycline with 250 mg. of glucosamine and 250,000 units of nystatin, bottles of 50. *Terrastatin for Oral Suspension,* each 5 cc. teaspoonful of reconstituted suspension contains 125 mg. of oxytetracycline with 125 mg. of glucosamine and 125,000 units of nystatin, 60 cc. bottles.

More detailed professional information available on request.

Pfizer *Science for the world's well-being®*
PFIZER LABORATORIES *Division, Chas. Pfizer & Co., Inc. New York 17, New York*

DIHYDROCODEINONE RESIN—PHENYLTOLOXAMINE RESIN

TUSSIONEX®

For the useless cough that debilitates!

A SINGLE DOSE CONTROLS COUGH FOR 8-12 HOURS with less narcotic and without preventing natural mucus discharge. 'Strasionic' release means *sustained* relief for "flu" cough or *any* cough—mild or severe. Two ounces last 6 days and nights!

TWO FORMS: Tussionex Thixaire™ Suspension • Tussionex Tablets.

FORMULA: Each teaspoonful (5cc.) or tablet contains 5 mg. dihydrocodeinone and 10 mg. phenyltoloxamine as cation exchange resin complexes of sulfonated polystyrene. *Warning: Dihydrocodeinone may be habit forming.*

INDICATIONS: Cough, including those due to influenza, colds, bronchitis, chronic sinusitis, pharyngitis, chronic lung disease, cardiac decompensation, measles.

DOSE: 1 teaspoon or tablet q 12 h. Children under 1 year, ¼ teaspoon q 12 h; 1-5 years, ½ teaspoon q 12 h.

EFFECTS: 8-12 hour cough suppression.

SIDE EFFECTS: Negligible, but when encountered may include mild constipation, nausea, facial pruritis, drowsiness, which disappear with adjustment of dose or discontinuance of treatment.

OVERDOSAGE: Immediately evacuate the stomach. Respiratory depression, if any, can be counteracted by respiratory stimulants. Convulsions, sometimes seen in children, can be controlled by intravenous administration of short-acting barbiturates. Hypothermia can be controlled by the usual supportive methods.

Rx only: Class B taxable narcotic

FOR SUSTAINED RELIEF FROM COUGH DUE TO FLU

STRASENBURGH LABORATORIES
ROCHESTER, NEW YORK, U.S.A. DIV. WALLACE & TIERNAN INC.

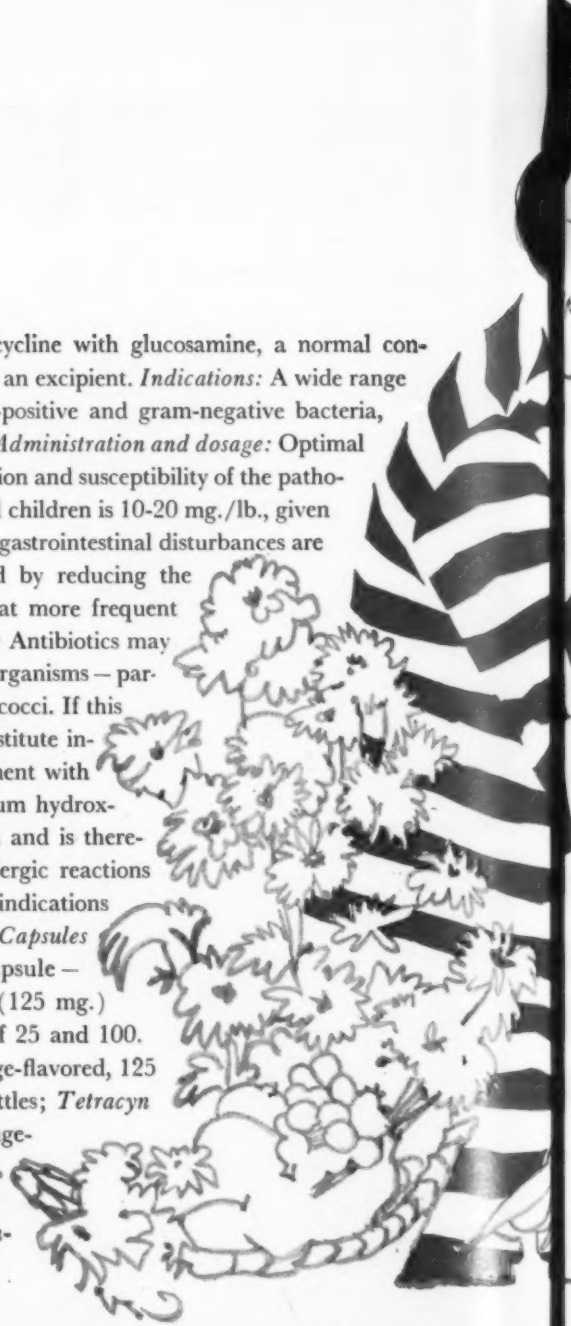
Originators of 'Strasionic' (sustained ionic) Release

So many infections are susceptible to **Tetracyn**

TETRACYCLINE WITH GLUCOSAMINE

Pediatric Drops Capsules Syrup

classic broad-spectrum antibiotic therapy



IN BRIEF Tetracyn provides tetracycline with glucosamine, a normal constituent of human tissues and fluids, as an excipient. *Indications:* A wide range of infections due to susceptible gram-positive and gram-negative bacteria, rickettsiae, large viruses and protozoa. *Administration and dosage:* Optimal dosage varies with the severity of infection and susceptibility of the pathogen. Average daily dose for infants and children is 10-20 mg./lb., given in divided doses at 6-hour intervals. If gastrointestinal disturbances are encountered, they may be minimized by reducing the individual dose and administering it at more frequent intervals. *Side effects and precautions:* Antibiotics may allow overgrowth of nonsusceptible organisms – particularly monilia and resistant staphylococci. If this occurs, discontinue medication and institute indicated supportive therapy and treatment with other appropriate antibiotics. Aluminum hydroxide gel decreases antibiotic absorption and is therefore contraindicated. Glossitis and allergic reactions are rare. There are no known contraindications to glucosamine. *Supplied: Tetracyn Capsules* (black-and-white), 250 mg. per capsule – bottles of 16 and 100. Half-strength (125 mg.) capsules (black-and-white) – bottles of 25 and 100. *Tetracyn Syrup – preconstituted,* orange-flavored, 125 mg./tsp. (5 cc.), 2 oz. and 1 pt. bottles; *Tetracyn Pediatric Drops – preconstituted,* orange-flavored, 100 mg./cc. (5 mg./drop), 10 cc. bottle with calibrated plastic dropper. *More detailed professional information available on request.*

Pfizer *Science for the world's well-being®*

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc. New York 17, New York

rompt antibacterial action and a
oad range of antibacterial
ctiveness with a remarkably low
egree of toxicity."[1]

[1] Mathieu, P. L., Jr., et al.: Rhode Island M.J. 42:172, 1959.

"why, he's breathing easier already, Doctor!"

In many instances, within five to ten minutes after you have administered Elixophyllin, the fight for air dramatically relents—spastic bronchioles relax, audible wheezing and rales disappear, vital capacity increases, normal breath sounds reappear and the anxiety of the child and parents fades away.

In 10 to 30 minutes, even severe attacks are usually terminated and mucus plugs become more easily expelled. Theophylline is inherently long acting and relief is *sustained* for hours

And there is no needle fright with Elixophyllin. This *oral* theophylline is so quickly absorbed that gastric irritation is seldom encountered and bronchospasmolytic (I.V.) theophylline blood levels are obtained in 15 minutes in the majority of cases.

No ephedrine-induced anxiety or other sympathomimetic side effects.

ELIXOPHYLLIN® ORALLY

BRONCHIAL ASTHMA and wheezing due to bronchospasm during respiratory infection—"asthmatic bronchitis."

PRECISION DOSAGE
effective, safe

ACUTE, SEVERE ATTACKS:

0.5 cc. per lb. body weight (if no theophylline in any form has been given in preceding 12 hours). Dehydration, prolonged hypoxia and status episodes may often be prevented by use early in attack. Do not repeat dose in less than 6 hours. Two such doses may be given every 24 hours if needed.

24 HOUR THERAPY:

First 6 doses 0.3 cc. per lb. body weight. Thereafter, 0.2 cc. per lb. body weight.

Administer before breakfast, at 2 P.M. and at bedtime on empty stomach. Sweeten doses if desired.

Whenever steroids must be given, concurrent administration of Elixophyllin in 24 hour control dosage usually reduces steroid requirements and side effects, and facilitates their withdrawal.

Each cc. of Elixophyllin contains:
theophylline.....5.3 mg.
(aminophylline equivalent.....6.6 mg.)
vehicle contains: alcohol.....0.2 cc.
and sugars 33 mg.

At all pharmacies. Rx only.

Send for 3 oz. plastic bottle of Elixophyllin for your bag and reprints on experience with Elixophyllin in pediatrics.

Sherman Laboratories
Detroit 11, Michigan

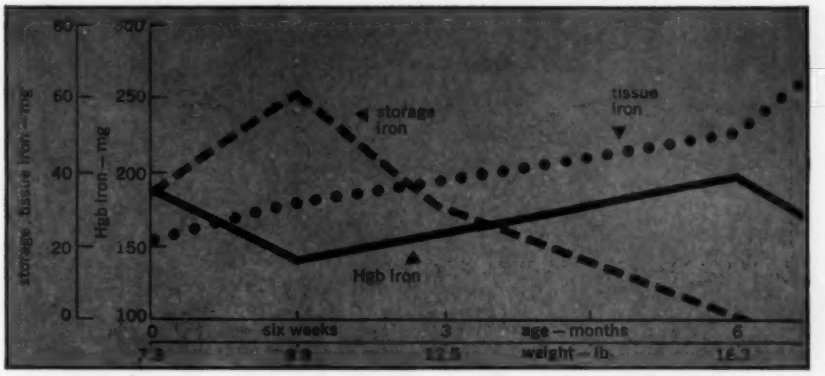
Patent Pending

iron in infancy

A STEADILY DIMINISHING RESERVE

When blood formation is resumed at 2 to 3 months of life, "the iron reserve again is utilized and supplies the needs for blood formation until age 6 months when stores again are exhausted. In absence of exogenous iron, a second fall in hemoglobin mass will occur as demands for tissue iron continue with growth."[1]

CHANGES IN IRON COMPARTMENTS OF THE AVERAGE TERM INFANT DURING THE FIRST YEAR[1]



"Iron deficiency . . . may occur during later infancy, even if the diet seems fairly adequate. . . . Even babies with excellent diets and adequate hemoglobin concentration show many indices of iron depletion."[1]

IRON DEPLETION PREVENTED WITH SOLID FOODS AND

SIMILAC WITH IRON®

12 mg of ferrous iron per quart of feeding

Feeding the usual diet of solid foods and Similac With Iron assures a total iron intake of at least 1.5 mg/kg body weight daily. This is the level recommended[2] for meeting current growth needs during the first year and for providing stores to prevent the common second-year depletion.

A group of infants fed Similac With Iron did not develop iron deficiency.[3] Among 74 healthy term infants and 42 prematures fed Similac With Iron and two control feedings, it was found that:

"The infants fed . . . [Similac With Iron] had higher values for hemoglobin, hematocrit and serum iron after 3 to 3½ months of age, and these values continued to be significantly higher during the 9-month period of observation."[3]

by 4 months of age
or by 14 pounds in weight

RESEARCH · SERVICE · NUTRITION · Ross

ROSS LABORATORIES, Columbus 16, Ohio

1. Schulman, I.: J.A.M.A. 175:118 (Jan. 14) 1961. 2. American Academy of Pediatrics Committee on Nutrition: Pediatrics 26:715 (Oct.) 1960. 3. Marsh, A. K., et al.: Pediatrics 24:404 (Sept.) 1959.

IRON: often a minus in moms and minors...

LIVITAMIN®

. . . the hematinic with built-in nutritional support

Many growing children and most women of menstrual age deplete their iron reserves and slide into iron-deficiency anemia.

Livitamin changes the minus to a plus because it restores depleted iron reserves and also provides integrated nutritional support.

Iron in Livitamin is well absorbed, with minimum gastric upset and constipation. And with Livitamin there is no worry about teeth stain . . . or taste acceptance.

WRITE FOR LITERATURE
AND DOSAGE INFORMATION.

FORMULA: Each fluidounce contains:

Iron, peptonized (equiv. in elemental iron to 71 mg.)	420 mg.
Manganese citrate, soluble, N.F.	158 mg.
Thiamine hydrochloride	10 mg.
Riboflavin	10 mg.
Cobalamin	20 mcg.
Nicotinamide	50 mg.
Pyridoxine hydrochloride	1 mg.
Pantothenic acid	5 mg.
Liver fraction 1	1 Gm.
Rice bran extract, U.S.P. XIV	1 Gm.
Inositol	30 mg.
Choline	60 mg.

SUPPLIED: *Liquid:* 8 oz. bottles, pints, gallons; *Capsules:* Bottles of 100, 500, 1000. Also available as LIVITAMIN with INTRINSIC FACTOR: bottles of 100 capsules.

THE S. E. MASSENGILL COMPANY

Bristol, Tennessee • New York • Kansas City • San Francisco

Eliminate

PINWORMS
ROUNDWORMS

Without staining • vomiting • enemas
nausea • fasting • laxatives

Available as...

'ANTEPAR' SYRUP Piperazine Citrate, 100 mg. per cc.

'ANTEPAR' TABLETS Piperazine Citrate, 500 mg., scored

'ANTEPAR' WAFERS Piperazine Phosphate, 500 mg.

Literature and patient instruction sheets available on request

BURROUGHS WELLCOME & CO. (U.S.A.) INC., Tuckahoe, New York

The principle is
entrapped flavor

The product is
new Vi-Daylin
Chewable

The result is a tablet that's "candy" all the
way through (you can't even taste the riboflavin)

Profile of a multivitamin

New Vi-Daylin Chewable —with entrapped flavor

New Formula

In recognition of recent medical thinking, we've reduced the vitamin D in our formula from 20 mcg. (800 units) to 10 mcg. (400 units). At the same time, we've increased the vitamin C content from 40 mg. to 50 mg. per tablet and per 5-cc. lemon-candy teaspoonful.

All Other Elements Remain at Their Previous Level.

Vitamin A (3000 units)	0.9 mg.
Thiamine Mononitrate	1.5 mg.
Riboflavin	1.2 mg.
Cobalamin (B_{12})	3 mcg.
Nicotinamide	10 mg.
Pyridoxine Hydrochloride	1 mg.

New Low Price

In quantities of 100 tablets our new Chewable costs less than 4¢ a tablet and the normal dosage is one tablet daily. No financial hardship for your patients when you prescribe or recommend Vi-Daylin.

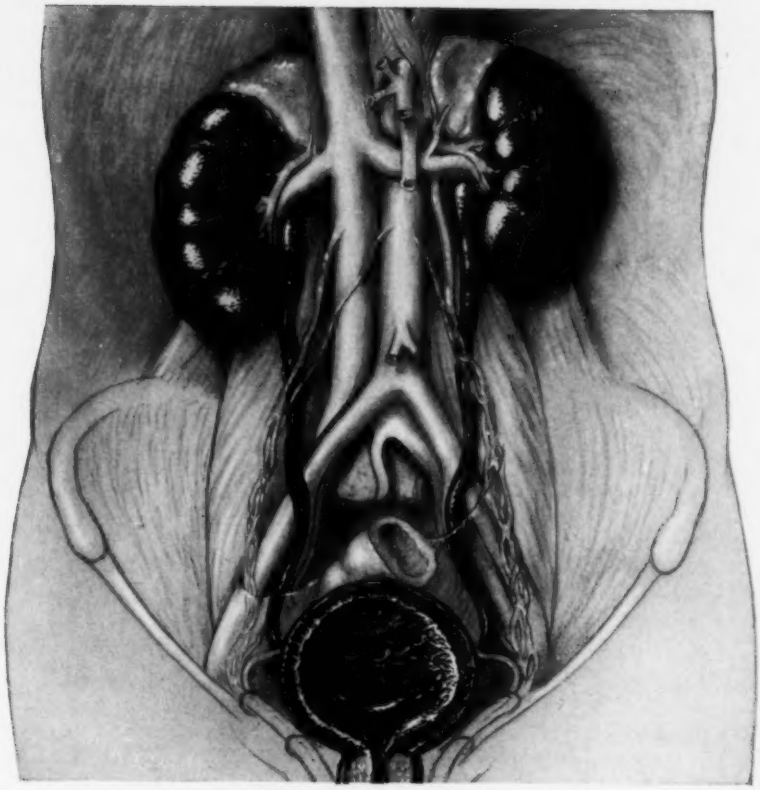
New Shape, New Color, New Bottle

New Vi-Daylin Chewable tablets are football shaped. This shape got a high degree of acceptance in our taste-tests and seems to have an intrinsic interest for children. The orange color ties in with the mild, sweet citrus flavor. And the wide-mouthed new bottle looks handsome on the table.

Taste-Test New Vi-Daylin Chewable Yourself

Won't you taste-test new Vi-Daylin Chewable multivitamins yourself? We're certain you'll be pleasantly surprised at their sweet good taste. They're the candy-flavored multivitamins with . . . little folks taste the candy flavor, never the vitamins.

Vi-Daylin—Vitamins A, D, B_1, B_2, B_6, B_{12}, C, and Nicotinamide, Abbott



Why a triple sulfonamide?

SPECTRUM— that encompasses certain common bacteria not susceptible to antibiotics, such as gram-negative bacteria of the urinary tract.

EFFICACY— in many genito-urinary infections. In upper respiratory infections and genito-urinary infections, active at the foci of infection. May succeed where bacteria are resistant to antibiotics. Rapid bacteriostatic effect.

SAFETY— safer than a single sulfonamide. Independent solubilities of the three sulfonamide components minimize danger of crystalluria. Fewer of the complications of antibiotic therapy such as allergic reactions, diarrhea, gastrointestinal upset, superinfection.

ECONOMY— lower cost than with most antibiotic prescriptions.

SUSPENSION TABLETS

SULFOSE®

Triple Sulfonamides, Wyeth
(Sulfadiazine, Sulfamerazine, Sulfamethazine)

For further information on limitations, administration, and prescribing of SULFOSE, see descriptive literature or current Direction Circular.

Wyeth Laboratories
Philadelphia 1, Pa.

Wyeth® SERVICE TO MEDICINE

ERYTHROCIN Stearate is—and has been—considered one of the safest antibiotics in practice. After millions of prescriptions, *there hasn't been a single report implicating it with damage to the kidneys . . . the liver . . . or the blood-forming organs.*

But Erythrocin is more than just a safe antibiotic. It's also a potent agent with bactericidal activity against many of the everyday infections you encounter. Whenever a coccal infection is involved—*and this includes many staphylococci that have developed resistance to penicillin and the tetracyclines*—Erythrocin may well be considered the drug of choice.

When it comes to patient-sensitivity to penicillin, many physicians consider this point alone an important reason for prescribing Erythrocin. Allergic reactions may occur, of course. But sensitivity to Erythrocin is so rarely encountered *that it is seldom considered an obstacle to treating patients.*

You'll find Erythrocin available in a complete line of dosage forms—*including the only intramuscular product in the erythromycin field*. The Filmtab® tablets come in two compact sizes, 100 and 250 mg. There's also a ready-mixed, citrus-flavored oral suspension, a form for intravenous use and even a combination with the triple sulfas.

®Filmtab—Film-sealed tablets, Abbott

ABBOTT

FOR SAFETY... FOR EFFECTIVENESS

(Erythromycin Stearate, Abbott)

Erythrocin®

STEARATE

109200

combating bacterial infections in children? *consider these 8 clinically significant advantages of TERFONYL® Squibb Triple Sulfas (Trisulfapyrimidines) in highly palatable suspension*

TERFONYL rarely causes superinfection TERFONYL soluble in urine at varying pH TERFONYL minimal disturbance of intestinal flora TERFONYL rarely sensitizes TERFONYL effective against wide range of gram-positive and gram-negative pathogens TERFONYL diffuses rapidly through tissues TERFONYL readily crosses blood-brain barrier TERFONYL sustains therapeutic blood levels and TERFONYL is economical, too. Supply: Suspension, raspberry flavored, 0.5 Gm. per teaspoonful (5cc.). Also Tablets, 0.5 Gm. Each teaspoonful, each Tablet, provides 0.167 Gm. each of sulfadiazine, sulfamerazine, and sulfamethazine. For full information see your Squibb Product Reference or Product Brief.

SQUIBB *Squibb Quality—the Priceless Ingredient*

Terfonyl® is a Squibb trademark

Announcing a NEW member of the MULVIDREN family.. MULVIDREN DROPS for infants. Pleasant tasting Orange-Vanilla MULVIDREN DROPS are specially formulated to provide latest authoritative, safe levels of the vitamins needed for modern infant feeding.

MULVIDREN DROPS®

Each 0.6 cc. contains:
VITAMINS: A...3000 USP Units; D...400 USP Units; C...60 mg.; B_1...1 mg.; B_2...1.2 mg.; B_6...0.5 mg.; B_{12}...1 mcg. and Niacinamide...10 mg.

DOSAGE: Infants 0.3 cc. on the tongue or in the formula. Older Children 0.6 cc.

AVAILABLE: Bottles of 30 cc. at all pharmacies.

When formula days are over

and for all the other members of the family

pleasant tasting MULVIDREN® in SOFTAB® form

melts in the mouth—no water needed—the multivitamin EVERYBODY likes

EACH TABLET CONTAINS:
VITAMINS: A...5000 USP Units; D...1000 USP Units; C...75 mg.; B_1...2 mg.; B_2...2 mg.; B_6...1.2 mg.; B_{12} (as Ionex-12*), 3 mcg.; d-Calcium Pantothenate...3 mg.; Niacinamide...10 mg.

DOSAGE: 1 tablet daily or more as directed by physician.

AVAILABLE: Bottles of 50 & 100 tablets at all pharmacies.

*Stuart Ionex-12 increases Vitamin B_{12} absorption.

THE STUART COMPANY Stuart PASADENA, CALIFORNIA

12688/4101

BUT DOCTOR, AREN'T CALCIUM TABLETS AS GOOD AS MILK?

NO . . . calcium tablets are *not* as good as milk . . . because in providing the body with essential nutrients milk is really calcium-*plus*. Milk and other dairy foods supply most of the calcium in the diet, and contribute protein, riboflavin and many other nutrients that are needed daily by the healthy adult, the older citizen, the overweight and the patient with a disturbance of calcium metabolism. All need the liberal amounts of these ingredients . . . as well as calcium . . . with which milk is uniquely endowed. And for those on a restricted diet, inclusion of dairy and other foods which are rich in the nutrients that are sometimes marginal in our diet . . . is especially important.

When consumed in suggested amounts . . . two glasses daily for adults . . . milk or its equivalent in milk products provides two-thirds of the 0.8 gram of calcium recommended for maintenance, plus two-fifths of the riboflavin, one-fourth of the protein and important amounts of vitamin A, thiamine and other nutrients. If credited with the value of the other nutrients it contains, milk is by far the most economical source of calcium available.

During the middle and later years of life, changes take place in the body which may have an adverse effect on calcium metabolism. The formation of stones containing calcium and other salts which may block the urinary tract is affected by several known factors including sex, race, and geography. The consumption of recommended amounts of *dietary calcium* has not been found to be associated with the development of *urinary calculi*. In some cases of osteoporosis . . . the most common bone disease in women of middle age . . . a liberal calcium allowance may be a valuable adjunct to hormonal therapy in stimulating recalcification of demineralized bone!

Milk and other dairy foods stand alone in their ability to provide calcium needed by adults of all ages in a form that is highly palatable, easily digested, and readily absorbed . . . and they also provide other nutrients which contribute to a balanced dietary and buoyant health.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

AVAILABLE ON REQUEST:
Reprints of this series of messages on Calcium
NEW CALORIE-RESTRICTED DIET SHEETS

A non-profit organization

Since 1915 promoting better health through nutrition research and education

NATIONAL DAIRY COUNCIL
111 NORTH CANAL STREET • CHICAGO 6, ILLINOIS

Schering

"The experience to date with griseofulvin has been so promising for the management of *Microsporum audouini, Trichophyton tonsurans* and *Trichophyton violaceum* that it has become the treatment of choice for these infections of the scalp."[1]

oral
fulvicin®
griseofulvin

Supplied: FULVICIN Tablets (scored), 500 mg., in bottles of 20 and 100; 250 mg., in bottles of 30, 100 and 500. *Reference:* Sulzberger, M. B., *et al.*: Dermatology: Diagnosis and Treatment, ed. 2, Chicago, Year Book Publishers, 1961, p. 350. For complete details, consult latest Schering literature available from your Schering Representative or Medical Services Department, Schering Corporation, Bloomfield, N. J.

SCHERING CORPORATION • BLOOMFIELD, NEW JERSEY

S-828

Tinea capitis due to T. tonsurans *cleared after 7 weeks of therapy with* FULVICIN.

FROM WEINER, M. A.; GOULD, A. N., AND GANT, J. O., JR.: GRISEOFULVIN IN RINGWORM INFECTIONS. SCIENTIFIC EXHIBIT PRESENTED AT A.M.A. CLINICAL MEETING, DECEMBER, 1960, WASHINGTON, D. C.

for the diarrheal attack

effective–eradicates enteric bacterial pathogens

selective–does not eradicate the normal intestinal flora[1]

FUROXONE® LIQUID

brand of furazolidone

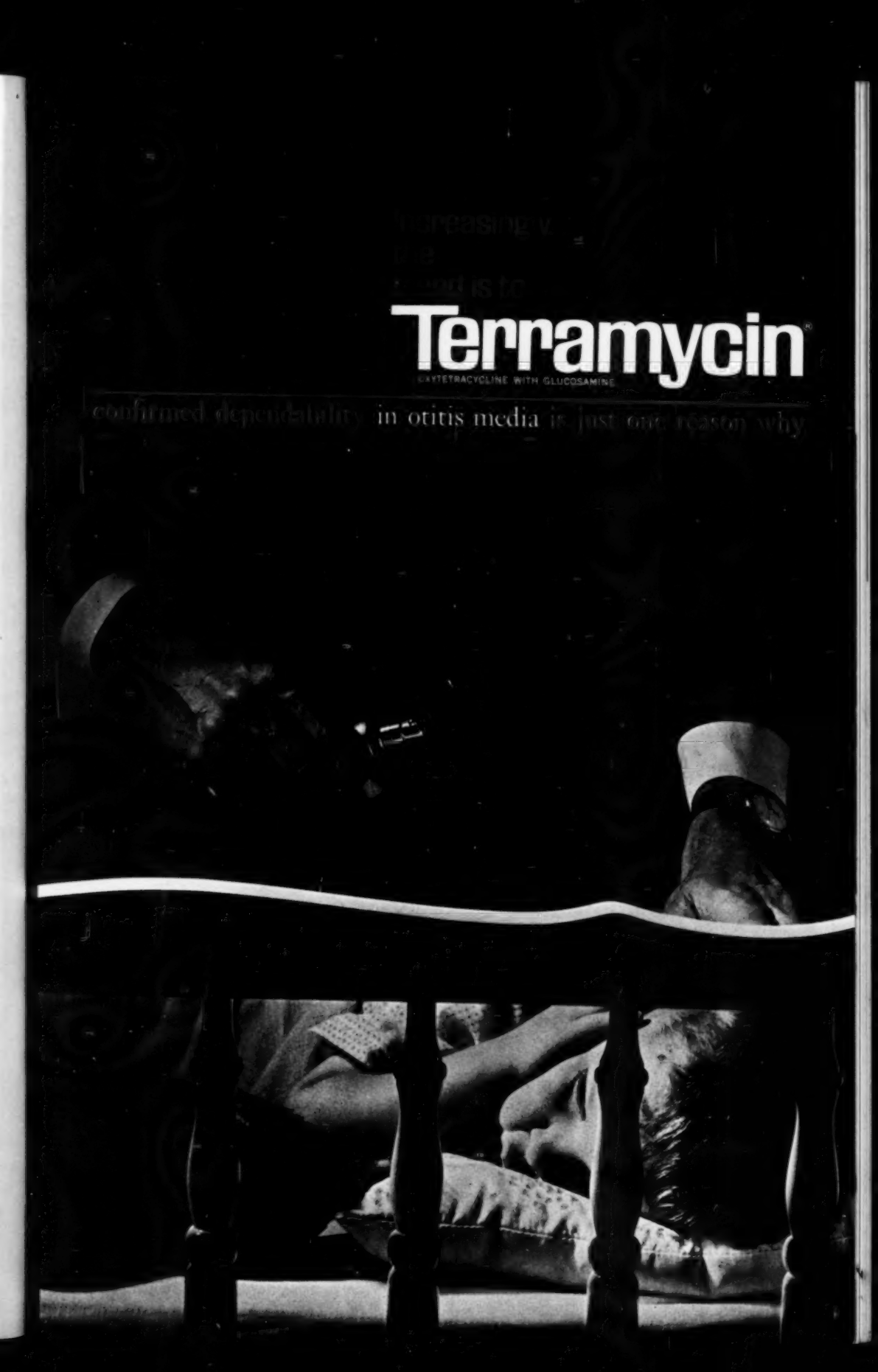
New, convenient prescription size: bottle of 2 oz. Also: bottle of 16 oz.

■ Exceptionally broad bactericidal range includes species and strains now resistant to other antimicrobials ■ Virtually nontoxic ■ Does not encourage monilial or staphylococcal overgrowth ■ Has not induced significant bacterial resistance ■ Dosage may be found in your PDR.

FUROXONE LIQUID is a pleasant orange-mint flavored suspension containing FUROXONE 50 mg. per 15 cc., with kaolin and pectin.

1. Mintz, A. A.: Antibiot. Med. 7:481, 1960.

EATON LABORATORIES, Division of The Norwich Pharmacal Company, NORWICH, N. Y.


Terramycin®
OXYTETRACYCLINE WITH GLUCOSAMINE
confirmed dependability in otitis media is just one reason why

*New evidence** demonstrates the effectiveness of Terramycin in *otitis media* . . . another reason for the trend to Terramycin.

In a series of 41 cases of otitis media, Terramycin not only "was often successful where other antibiotics had failed," but also showed that "it is extremely well tolerated"; oral dosage for infants was 250 to 375 mg. daily, for children, 500 mg. to 1 Gm. In many instances, oral therapy was preceded by intramuscular injection of Terramycin.

The authors concluded that "there is good reason to consider it [Terramycin] one of the most effective agents for treatment of infection of the upper respiratory tract."

These findings confirm the continuing vitality and broad-spectrum dependability of Terramycin, as reported through more than a decade of extensive clinical use.

In brief

The dependability of Terramycin in daily practice is based on its broad range of antimicrobial effectiveness, excellent toleration, and low order of toxicity. As with other broad-spectrum antibiotics, overgrowth of nonsusceptible organisms may develop. If this occurs, discontinue the medication and institute appropriate specific therapy as indicated by susceptibility testing. Glossitis and allergic reactions are rare. Aluminum hydroxide gel may decrease antibiotic absorption and is contraindicated.

More detailed professional information available on request.

Terramycin®

OXYTETRACYCLINE WITH GLUCOSAMINE

SYRUP PEDIATRIC DROPS

125 mg. per tsp. and 5 mg. per drop (100 mg./cc.), respectively

deliciously fruit-flavored aqueous dosage forms—conveniently preconstituted

another reason why the trend is to Terramycin—*versatility of dosage form:*

TERRAMYCIN Capsules—
250 mg. and 125 mg. per capsule—for convenient initial or maintenance therapy in adults and older children

TERRAMYCIN Intramuscular Solution—
50 mg./cc. in 10 cc. vials; 100 mg. and 250 mg. in 2 cc. ampules—preconstituted, ready to use where intramuscular therapy is indicated

Science for the world's well-being® Pfizer

PFIZER LABORATORIES Division, Chas. Pfizer & Co., Inc.
New York 17, N. Y.

*Jacques, A. A., and Fuchs, V. H.: J. Louisiana M. Soc. 113:200, May, 1961.

Schering

infantile eczemas, big problem of little patients, controlled with METI-DERM® AEROSOL

prednisolone topical

...and so are all other skin lesions responsive to topical steroids

available in 50 Gm. and 150 Gm. spray containers [illegible]

how would you design a tranquilizer specifically for children?

wouldn't you want it to be: | **see how closely these ATARAX advantages meet your standards:**

efficacious — "...Atarax appeared to reduce anxiety and restlessness, improve sleep patterns and make the child more amenable to the development of new patterns of behavior...."[1]

remarkably well tolerated — "The investigators were impressed with the lack of toxicity and minimal side effects which were observed even after long-term use."[2]

palatable — Delicious ATARAX syrup pleases even the balkiest patient.

Dosage: For children: under 6 years, 50 mg. daily; over 6 years, 50-100 mg. daily; in divided doses. For adults: 25 mg. t.i.d. to 100 mg. q.i.d. *Supplied:* Tablets 10 mg. and 25 mg., in bottles of 100 and 500. Tablets 100 mg., in bottles of 100. Syrup, 2 mg. per cc., in pint bottles. Also available: Parenteral Solution. Prescription only.

References: 1. Freedman, A. M.: Pediat. Clin. North America *5*:573 (Aug.) 1958. 2. Nathan, L. A., and Andelman, M. B.: Illinois M. J. *112*:171 (Oct.) 1957. 3. Santos, I. M. H., and Unger, L.: Ann. Allergy *18*:179 (Feb.) 1960. 4. Litchfield, H. R.: New York J. Med. *60*:518 (Feb. 15) 1960.

ATARAX® PASSPORT TO TRANQUILITY

(brand of hydroxyzine)

New York 17, N. Y.
Division, Chas. Pfizer & Co., Inc.
Science for the World's Well-Being®

VITERRA® Capsules—Tastitabs®
—Therapeutic Capsules for vitamin-mineral supplementation

BASIC!

in ear, nose and throat therapy

These six medications have demonstrated through clinical trials in countless patients, outstanding qualities of effectiveness and safety—qualities that have led to White's pre-eminence as a manufacturer of specialties for E. N. T. therapy. The pages that follow are a concise reminder of the clinical advantages offered by each. Complete information concerning the use of these specialties is available on request.

WHITE LABORATORIES, INC. / Kenilworth, New Jersey

Otobiotic®

3.5 mg. neomycin (from sulfate) and 50 mg. sodium propionate per cc.

Antibiotic/Antifungal EAR DROPS
"...Better results than ever before..."* in OTITIS EXTERNA & CHRONIC OTITIS MEDIA

• controls infection • reduces exudation • stops pruritus • physiologic pH • relieves pain • does not distort otic landmarks • virtually nonsensitizing and nonirritating

Available in 15 cc. dropper bottles.

*Lawson, G. W.: Postgrad. Med. 22:501 (Nov.) 1957

Dīsomer®

SYRUP

YOUR ALLERGIC PATIENT REMAINS ASYMPTOMATIC AND ALERT!

Aspergum®

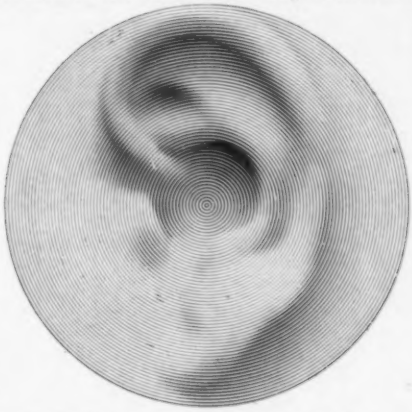
SOOTHES SORE THROATS

Available in handy pocket-packs of 16 and bottles of 36.

White **WHITE LABORATORIES, INC.** / Kenilworth, New Jersey

Otobione®

ANTI-INFLAMMATORY/Antibiotic/Antifungal Sterile EAR DROPS



Physiologic pH ■ Effective relief in external otitis, chronic otitis media and chronic mastoiditis with otorrhea. FORMULA: Prednisolone acetate, 5 mg., neomycin (from sulfate), 3.5 mg. and sodium propionate, 50 mg. per cc. Available in 5 cc. bottles with "steri-sealed" dropper.

Disophrol

In nasal congestion and rhinorrhea due to head colds, allergies and sinusitis—

Decongests Nasal and Sinus Passages...
Dries Mucous Secretions!

A sensibly simple formula: Disomer (dexbromphen-iramine maleate) 2 mg., d-isoephedrine sulfate 60 mg. Available in bottles of 100 scored tablets

Orabiotic®

chewing gum troches

IN POST-TONSILLECTOMY CARE

"Postoperative pain...was decidedly decreased... The tonsillar fossae...were uniformly cleaner..."*
• Stimulates salivary flow to keep oropharyngeal area moist and clean • Protects against infection and subsequent secondary hemorrhage
• Reduces local postoperative pain and muscle spasm • Speeds resumption of normal diet by lessening postoperative discomfort

FORMULA: Each troche contains Neomycin, 3.5 mg., Gramicidin, 0.25 mg., and Propesin, 2.0 mg. Always a useful adjunct to systemic therapy. Available in packages of 10 and 20.
*Rittenhouse, E. A.: E.E.N.T. Mo. 36:406, 1957.

White **WHITE LABORATORIES, INC.**/ Kenilworth, New Jersey

NEW
for the management of
ASTHMA

QUIBRON™

Theophylline-glyceryl guaiacolate, Mead Johnson ELIXIR

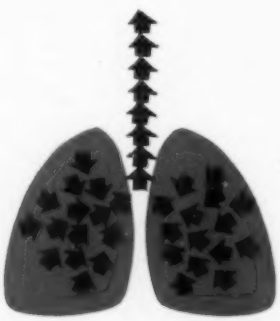
BRONCHODILATOR-EXPECTORANT

RELAXES CONSTRICTION by overcoming bronchospasm

Theophylline overcomes bronchospasm through relaxation of the bronchiolar smooth muscle.

REDUCES OBSTRUCTION due to tenacious mucus

Glyceryl guaiacolate increases respiratory tract fluids which dilute the thick tenacious mucus in the bronchioles, facilitating its removal and thereby reducing obstruction.



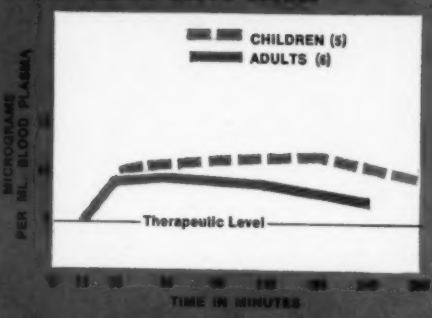
combines the bronchodilator effectiveness of theophylline[1-4]

with the proved expectorant action of glyceryl guaiacolate[5-8]

formulated for flexible low-volume dosage

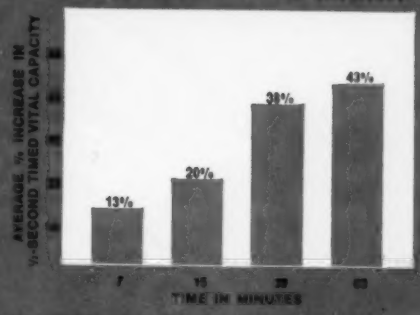
in a pleasant-tasting hydro-alcoholic vehicle for rapid absorption

QUIBRON RAPIDLY PROVIDES EFFECTIVE THEOPHYLLINE BLOOD LEVELS[1-4]



Following administration of Quibron elixir at the recommended dosage for adults[1] and children,[4] effective blood levels of theophylline were reached within 15-30 minutes and maintained for more than four hours.

THERAPY WITH QUIBRON IS FOLLOWED BY RAPID IMPROVEMENT OF VITAL CAPACITY[9]



Following administration of 20 ml. Quibron elixir, vital capacity was markedly improved, as measured by the timed ½-second method. Pulmonary function tests confirmed the clinical observation of rapid relief occurring in 10-15 minutes in most patients.[9]

Indications: Bronchial asthma, asthmatic bronchitis, chronic bronchitis and pulmonary emphysema.

Dosage & Administration: Adults: 1 to 2 tablespoons, 2-3 times daily. *Children, 6-12:* 1 tablespoon, 2-3 times daily. (Children weighing over 100 lbs. may require adult doses.) *Children under 6:* ½ teaspoon per 10 lbs. body weight, 2-3 times daily. During the first day of treatment, especially in severe attacks, the usual dose may be increased by one half.

Side Effects: Theophylline may cause gastric irritation, with possible abdominal discomfort, nausea and vomiting. The administration of Quibron elixir after meals may help avoid such symptoms. Theophylline may also exert some stimulating effect on the central nervous system.

Cautions: Quibron elixir should not be administered more frequently than every 6 hours or within 12 hours after rectal administration of any preparation containing theophylline or aminophylline. Other formulations containing xanthine derivatives should not be given concurrently with Quibron elixir. *Note:* Aminophylline toxicity has been prominent in the awareness of physicians who treat children. The use of theophylline in pediatrics has been thoroughly reviewed by Levin.[10] The predominance of theophylline toxicity reported in the pediatric literature has been in children treated with rectal forms of aminophylline or with a combination of rectal and oral dosage forms.[11]

Supplied: Each tablespoon of Quibron elixir (15 ml.) contains theophylline 150 mg. and glyceryl guaiacolate 90 mg. in a 15% hydro-alcoholic vehicle. Bottles of 8 fl. oz.

References: (1) Schlager, J., McGinn, J. T., and Hennessy, D. J.: Am. J. M. Sc. *233*:296-302 (March) 1957. (2) MacLaren, W. R.: California Med. *91*:278-282 (Nov.) 1959. (3) MacLaren, W. R.: Ann. Allergy *17*:729-739 (Sept.-Oct.) 1959. (4) Spielman, A. D.: Ann. Allergy *15*:270-276 (May-June) 1957. (5) Cass, L. J., and Frederick, W. S.: Am. Pract. & Digest Treat. *2*:844-851 (Oct.) 1951. (6) Schwartz, E., Levin, L., Leibowitz, H., and McGinn, J. T.: Am. Pract. & Digest Treat. *7*:585-588 (April) 1956. (7) Schiller, I. W., and Goldman, G.: Personal communication on file at the Mead Johnson Research Center.* (8) Levin, S. L., and Weisnagel, J.: Personal communication on file at the Mead Johnson Research Center.* (9) Pala, R. J., and Grates, W. C.: Current Therap. Res., in press (Nov.) 1961. (10) Levin, S. J.: Aminophylline (Theophyllitz) in Pediatrics; *Quarterly Review of Pediatrics 14*:107, 1959. (11) White, B. H., Daeschner, C. W.: Aminophylline (theophylline ethylene-diamine) poisoning in children. Journal of Pediatrics 49:262, 1956.

*These data are available to physicians on request.

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1. Newsome, C. K.: The challenge of triacetyloleandomycin in pediatric infections, J. Indiana M.A., *53*:1131 (June) 1960.

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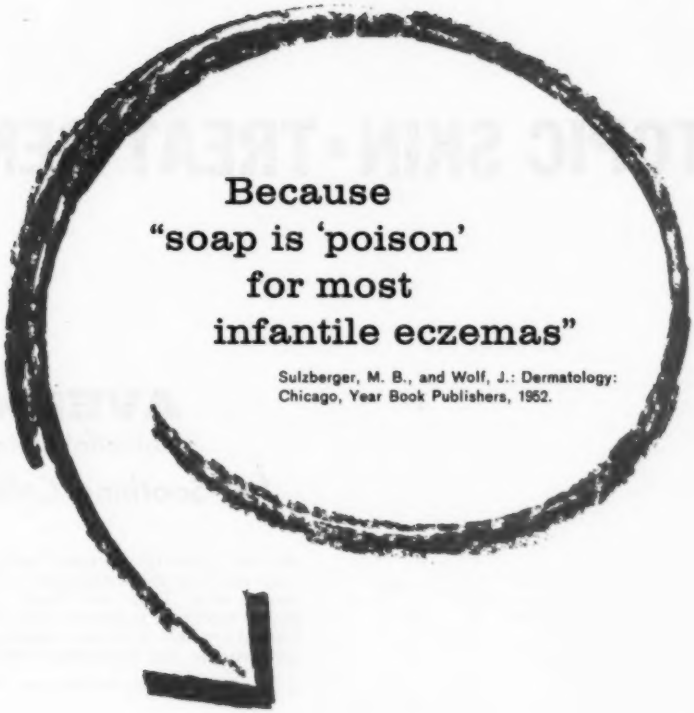

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References: 1.Epstein, N. N.: Eczema, Allergic, in Infants and Children (Atopic Dermatitis) : in Current Therapy, 1960. 2. Senear, F. E., and Haeberlin, J. B., Jr.: M. Clin. North America, March 1958. 3. Brunsting, L. A.: Postgrad. Med. 26:431, 1959.

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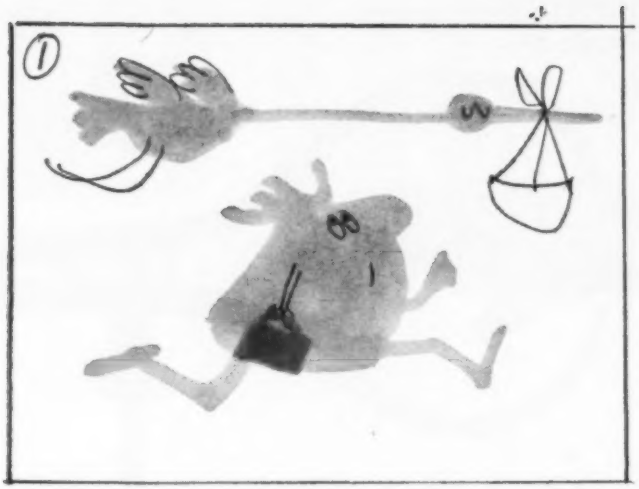
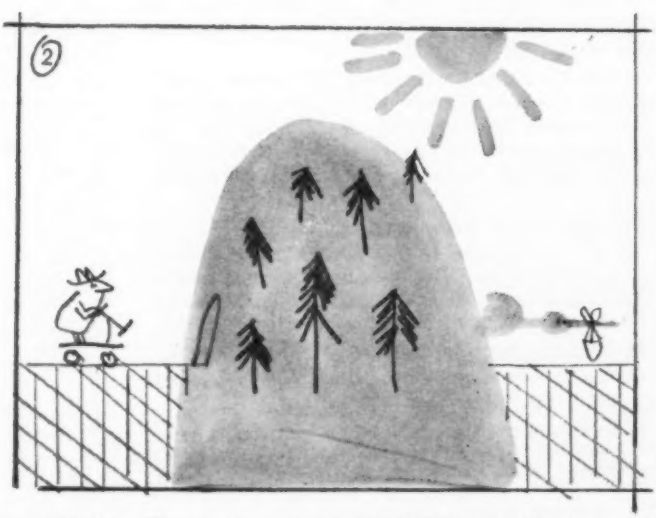
Bibliography: **1.** Nelson, W. E., in Textbook of Pediatrics, ed. 7, Nelson, W. E., ed., Philadelphia, W. B. Saunders Company, 1959, p. 1110. **2.** Bakwin, H.: J. Pediat. *14*:1 (Jan.) 1939. **3.** Gittleman, I. F. *et al.*: Pediatrics *18*:721 (Nov.) 1956. **4.** McCance, R. A., and Widdowson, E. M.: Acta Paediat. (Upps.) *49*:409 (July) 1960. **5.** Fomon, S. J., *et al.*: Pediatrics *23*:113 (Jan.) 1959. **6.** Bruck, E., and Weintraub, D. H.: Am. J. Dis. Child. *90*:653 (Dec.) 1955. **7.** Gittleman, I. F., and Pincus, J. B.: Pediatrics *8*:778 (Dec.) 1951. **8.** Talbot, N. B., *et al.*: Metabolic Homeostasis, Cambridge, Mass., Harvard University Press, 1959, pp. 110, 112, 117. **9.** Rubin, M. I., in Textbook of Pediatrics, ed. 7, Nelson, W. E., ed., Philadelphia, W. B. Saunders Company, 1959, p. 1035. **10.** Albright, F., *et al.*: Endocrinology *30*:922, 1942. **11.** Pincus, J. B., *et al.*: Ann. New York Acad. Sc. *64*:424 (Aug.) 1956. **12.** Weil, W. B., and Wallace, W. M.: Pediat. Clin. North America *6*:123 (Feb.) 1959. **13.** McCrory, W. W., and Macaulay, D.: Pediatrics *19*:639 (April) 1957. **14.** Barnett, H. L., and Vesterdal, J.: J. Pediat. *42*:99 (Jan.) 1953. **15.** American Academy of Pediatrics, Committee on Nutrition: Pediatrics *19*:339 (Feb.) 1957. **16.** Calcagno, P. L., and Rubin, M. I.: J. Pediat. *58*:686 (May) 1961. **17.** DiGeorge, A. M., and Warkany, J., in Textbook of Pediatrics, ed. 7, Nelson, W. E., ed., Philadelphia, W. B. Saunders Company, 1959, p. 1175. **18.** Payne, W. W.: Pediatrics *17*:84 (Jan.) 1956. **19.** Hillman, D. A., *et al.*: Pediatrics *21*:430 (March) 1958. **20.** McCrory, W. W., and Macaulay, D.: Pediatrics *19*:481 (March) 1957. **21.** Beaser, S. B.: Am. J. M. Sc. *213*:441 (April) 1947. **22.** Talbot, N. B., *et al.*: Functional Endocrinology, Cambridge, Mass., Harvard University Press, 1952, pp. 498-518, 528. **23.** Engle, M. A.: Pediat. Clin. North America *6*:241 (Feb.) 1959. **24.** Nadas, A. S.: Pediatric Cardiology, Philadelphia, W. B. Saunders Company, 1957, p. 247.



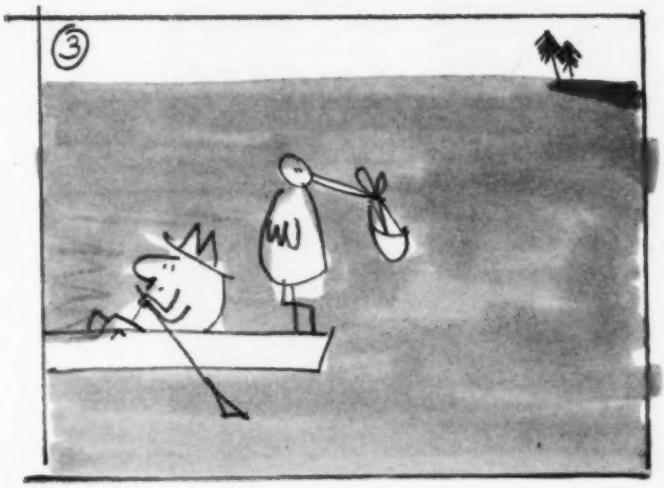
Hear about the new pediatric responsibility?

Nope. Faster, faster.



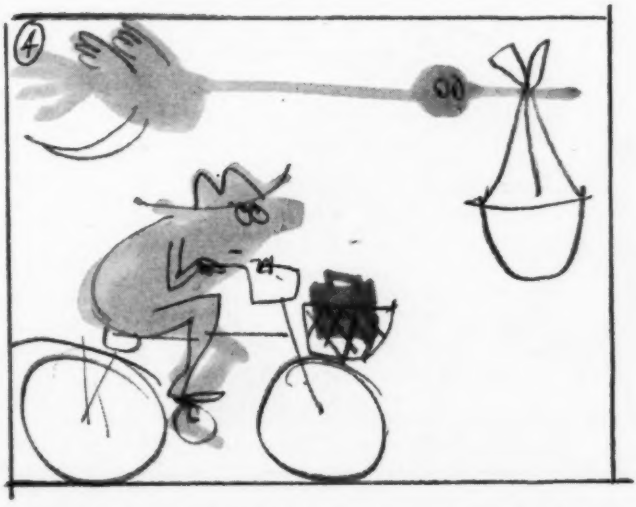
Teeth. They completely calcify at such an early age, lots of children don't get fluoride in time to effect maximum benefits.

Isn't that the dentist's area?



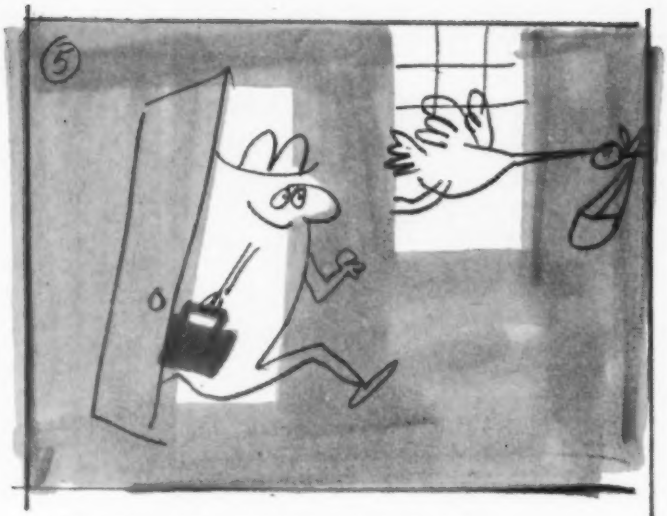
Trouble is many kids don't see the dentist soon enough. How many children under 4 go to the dentist?

Hmm. Faster, faster.



There's a new way to help insure adequate fluoride--take it right with the vitamins.

Good rationale. Faster!



Doesn't cost any more than plain vitamins either.

What age should babies start taking fluoride?

now is the time (the earlier the better) to protect his permanent teeth

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And—the younger the child at the time fluoridation is started, the greater the benefits. Because hard tooth substance begins to form at the embryonic age of about twenty weeks, fluoride should be administered as early in life as possible, if decay-resistant tooth enamel is to be achieved. *Adeflor supplies fluoride with routine pediatric vitamin supplementation and costs no more than vitamins alone.*

Note: The single lethal dose of sodium fluoride in the adult is between 5 and 10 Gm. On this basis, the lowest single lethal dose for a two-year old is 714 mg. This is more than 28 times the amount of fluoride supplied (25 mg.) in an entire 30 cc. bottle of Adeflor. *Adeflor used when and as recommended supplies safe fluoride supplementation.*

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REFERENCES: 1. Bessey, Otto A.; Adam, Doris J. D., and Hausen, Arild E.: Pediatrics *20*:33-34 (July) 1957. 2. Council on Dental Therapeutics, J. Am. Dental Assoc. *56*: 589-591 (April) 1958. 3. Expert Committee on Water Fluoridation, First Report, World Health Report Series, No. 146, 1958. 4. Nelson, Waldo E.: Textbook of Pediatrics, Philadelphia, W. B. Saunders, 1959, ed. 7.

Brief Basic Information

Formula:

Each 0.6 cc. of Adeflor Drops contains:

Vitamin A (Synthetic)	(5000 units) 1.5 mg.
Vitamin D_3	(1000 units) 25 mcg.
Ascorbic Acid (C)	50 mg.
Pyridoxine Hydrochloride (B_6)	1 mg.
Fluoride (as sodium fluoride)	0.5 mg.

Indications: As an aid in the prevention of dental caries and in the prophylaxis and treatment of deficiencies of Vitamins A, C, D, and B_6.

Dosage and Administration: Where the drinking water is substantially devoid of fluoride, infants and children may be given 0.6 cc. Adeflor Drops daily.

May be administered by dropping directly on the tongue or by mixing with water, milk, fruit juice, or food.

Precautions and Contraindications: The use of this product is recommended only in areas where the fluoride content of drinking water is known and is less than 0.7 parts per million.

Supply: 15, 30, and 50 cc. plastic bottles with a plastic, calibrated (0.6 cc.) dropper. Available on prescription only.

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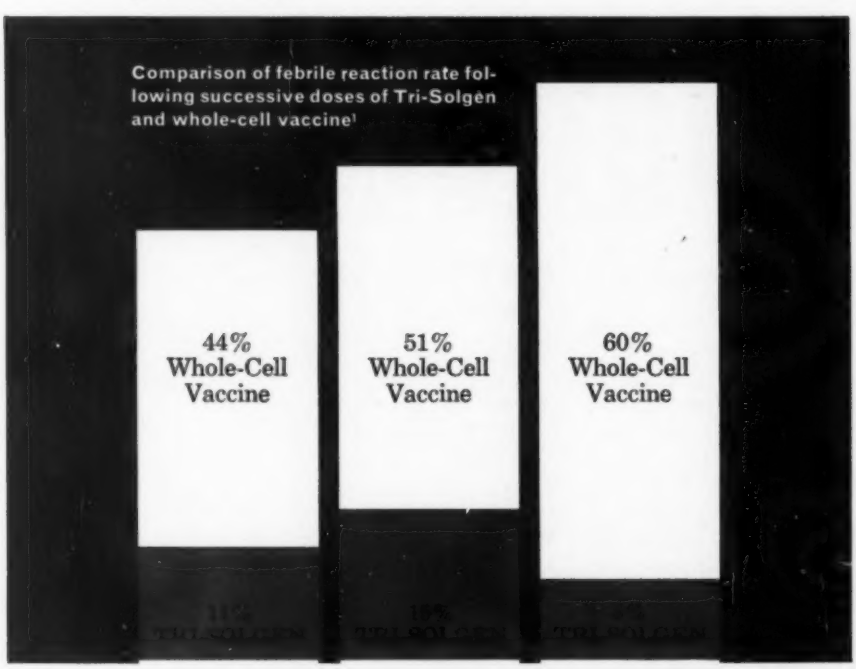
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44% Whole-Cell Vaccine
51% Whole-Cell Vaccine
60% Whole-Cell Vaccine

How Supplied: Tri-Solgen is available in 7.5-cc. vials (five immunizations).

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1. Weihl, C., Riley, H. D., Jr., and Lapin, J.: Scientific Exhibit, Meeting of American Academy of Pediatrics, October, 1960, and Interim Meeting of A.M.A., December, 1960.

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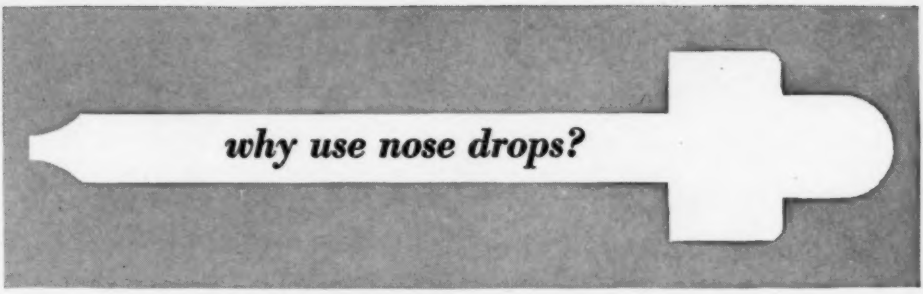

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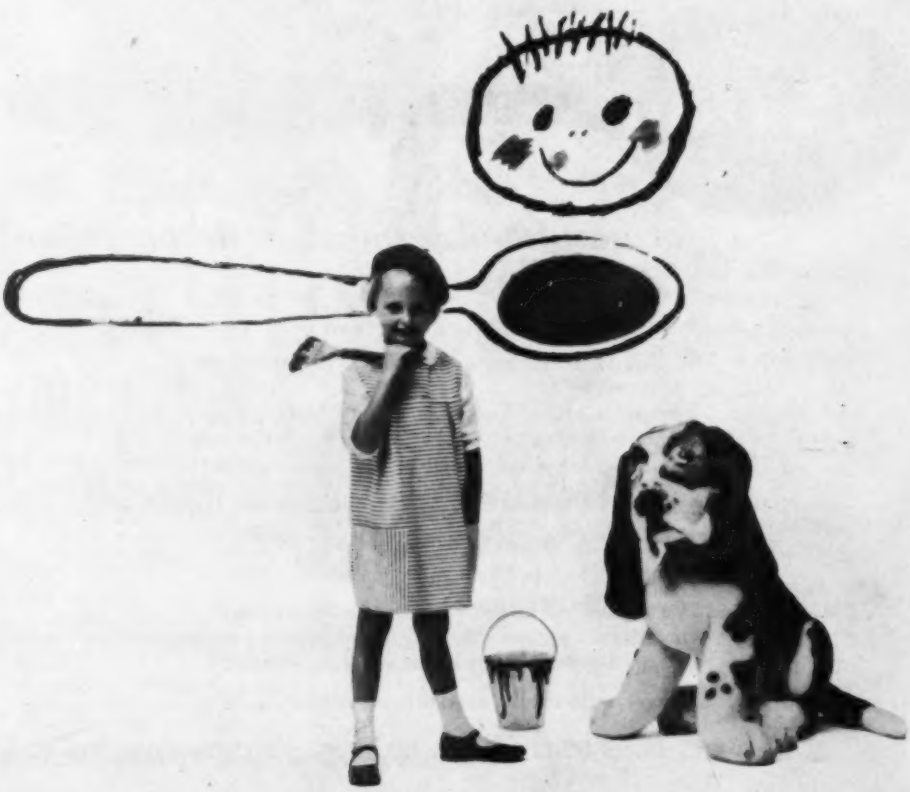

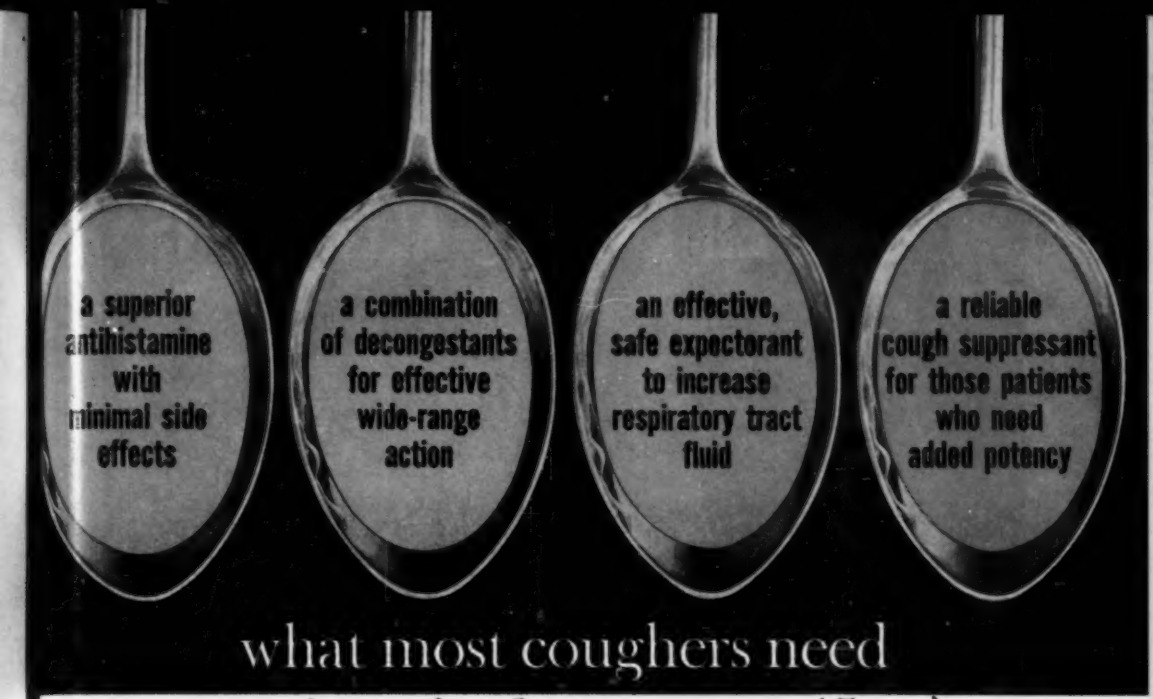
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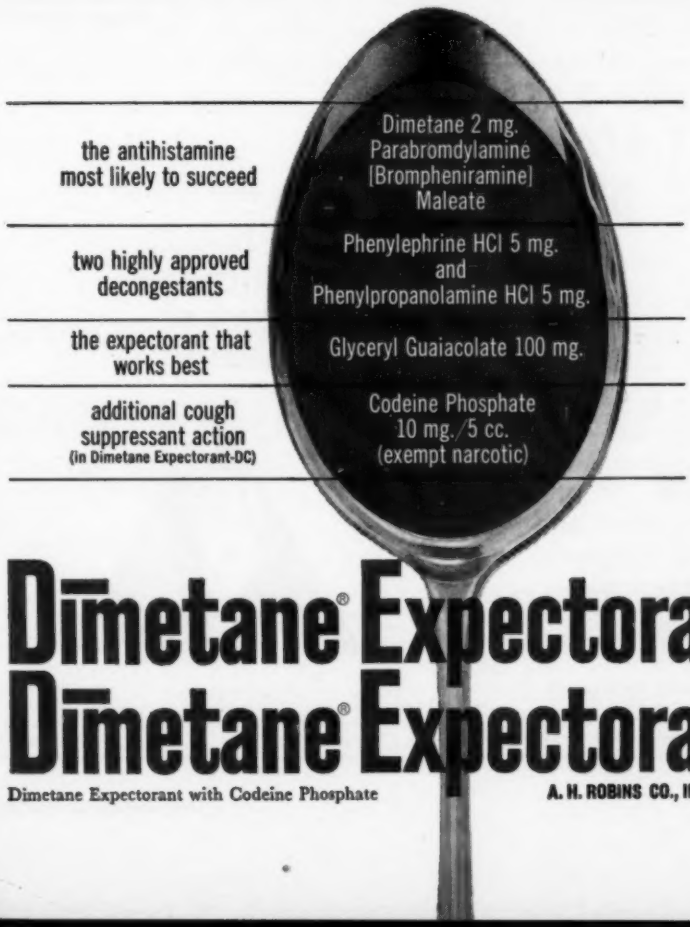
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1. Silbert, N. E., Ciba Clinical Symposia, 6: 86, May 1954
2. Mackenzie, J., Annals of Allergy, 12: 164, March 1954
3. Rosen, F. L., J. Med. Soc. N.J., 51: 110, March 1954

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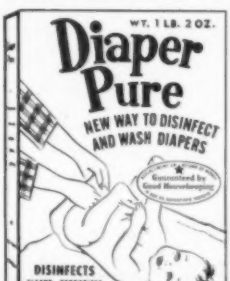

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- Disinfects, cleans, and deodorizes.

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1 package washes and treats 300 diapers.

ACTIVE INGREDIENTS: Trisodium Phosphate 3.5%, Sodium Metasilicate 3.5%, Triethanolamine Oleate 0.3%, N-Alkyl (60% C_{14}, 30% C_{16}, 5% C_{12}, 5% C_{18}) Dimethyl Benzyl Ammonium Chlorides 0.35%. Inert Ingredients 92.35%

Physicians' samples on request

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PEDIATRIC SUSPENSION
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SYRUP
(chocolate flavored)

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LIPO GANTRISIN
(vanilla-mint flavored)

Packages: GANTRISIN: *Pediatric Suspension:* Each teasp. (5 cc) provides the equivalent of 0.5 Gm Gantrisin, raspberry flavored—bottles of 4 oz and 16 oz. *Syrup:* Provides the equivalent of 0.5 Gm Gantrisin per teasp. (5 cc), chocolate flavored—bottles of 4 oz and 16 oz.

LIPO GANTRISIN® (the long-acting form of Gantrisin): Provides the equivalent of 1.0 Gm Gantrisin per teasp. (5 cc), vanilla-mint flavored homogenized mixture—bottles of 4 oz and 16 oz.

Consult literature and dosage information, available on request, before prescribing.

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Pleasantly flavored for ready acceptance. Highly adsorptive for prompt control of diarrhea. Contains CLAYSORB, with more than 5 times the adsorptive capacity of kaolin.

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restoration of normal absorption of fluids and nutrients
encouragement of normally soft, well-formed stools
palatable, low-dosage regimen

1. Barr, M., and Arnista, E.S.: J. Am. Pharm. A. (Scient. Ed.) *46*:493 (Aug.) 1957.
2. Bartell, P., Pierzchala, W., and Tint, H.: Am. J. Pharm. A. (Scient. Ed.) *49*:1 (Jan.) 1960.

For further information on limitations, administration, and prescribing of DIAMAGMA, see descriptive literature or current Direction Circular.

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1.) Council on Foods and Nutrition: J.A.M.A. 169:110 (Jan. 3) 1959. 2.) May, C. D.: Editorial Pediatrics 23:833 (May) 1959. 3.) Serbell, Jr., W. H.: Seminar Report 3:2 (Fall) 1958. 4.) Smith, D. W., et al.: Pediatrics 24:268 (Aug.) 1959. 5.) Gyorgy, P.: Mod. Med. 28:76 (Aug. 1) 1960. 6.) Sebrell, Jr., W. H.: Mod. Med. 28:79-80 (Aug. 1) 1960. 7.) Eagle, J. I.: New York J. Med. 60:1822 (June 1) 1960. 8.) Report of Joint Committee of American Academy of Pediatrics and American Society of Dentistry for Children: Pediatrics 23:403 (1959. 9.) Accepted Dental Remedies, American Dental Association, Chicago, 26th Ed., 1961, pg. 135. 10.) Arnold, Jr. F. A. McClure, F. J. and White, C. L.: Dental Progress 1:9-12 (October) 1960.

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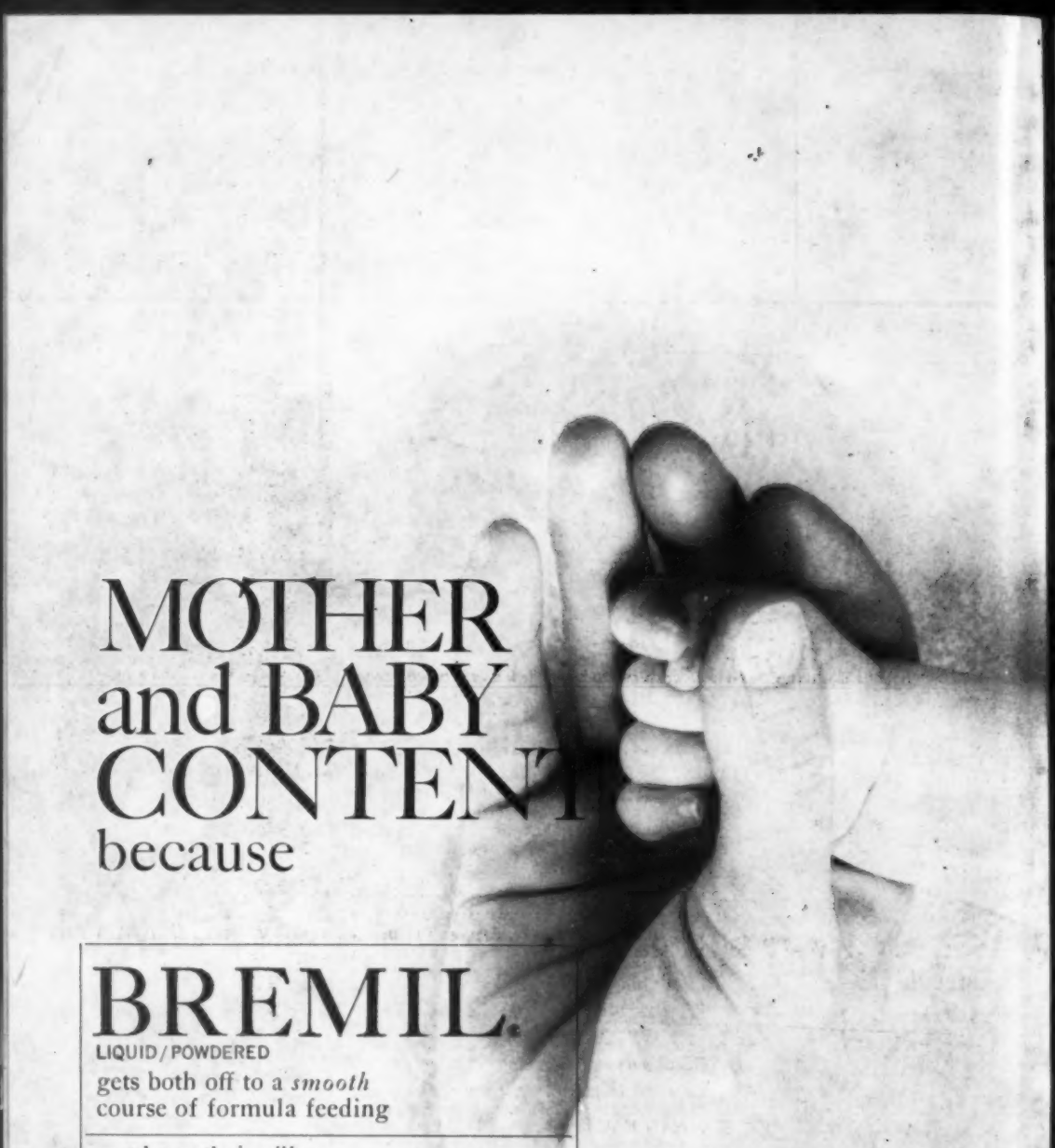

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